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Review Article

A brief review on genetics of opioid receptors in opioid addiction

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Abstract

Opioid addiction is a chronic mental illness that manifests itself through several relapses and remissions throughout the course of an addict's life. Over the last few decades, opioid addiction has developed into a significant public health epidemic. Classical molecular genetics research has limited the opioid receptor family down to three major subtypes: mu (μ), kappa (κ), and delta (δ) opioid receptors, which are encoded by the *OPRM1*, *OPRD1*, and *OPRK1* genes. Individuals' opioid addiction is regulated by this *OPRM1*, *OPRD1*, and *OPRK1* genes via a reward system route, including the dopaminergic system. Furthermore, when opioid ligands attach to these receptors, it produces euphoric and rewarding effects. Numerous research has been conducted on the single nucleotide variations (SNVs) of these genes in several ethnic groups.

The majority of the studies had focused on the *OPRM1* gene and its variants. Therefore, this article will summarize recent research on opioid receptor genetic variants associated with opioid addiction and emphasize the relevance and importance of investigating gene variants to ascertain genetic predisposition.

Keywords: *OPRM1*, *OPRD1*, *OPRK1*, opioid addiction, rs1799971

Introduction

Addiction to opioids is a significant public health problem that has reached epidemic proportions in many parts of the world. It is a chronic and multifaceted neurobiological disorder characterized by frequent relapses to the use of drugs (Kakko et al., 2019). In 2019, approximately 275 million (5.5%) of the world's population aged 15–64 years reported using drugs at least once during the year, where around 62 million of them have used opioids. In 2019, approximately 36.3 million people suffered from substance use disorders (WHO, 2021).

Addiction to opioids develops due to the use of illegal opioids such as heroin, morphine, or illicit prescription opioids as the treatment of chronic or acute pain. Following self-exposure, two major factors contribute to an individual's susceptibility to developing an opiate addiction. These are genetic factors, which account for 40%–60% of the variability in risk of developing an opioid addiction, the highest of any substance of abuse (Mura et al., 2013; Orna Levran et al., 2012; Kendler, Jacobson, et al., 2003) and the environment's influence on the individual (Prom-Wormley et al., 2017; Kendler et al., 2008). Opioids continue to bear the burden of the majority of drug-related diseases. Opioids are defined by their ability to bind to G protein-coupled opioid receptors mu (μ), kappa (κ), and delta (δ), which are encoded by distinct genes (*OPRM1*, *OPRK1*, and *OPRD1*) and are stimulated by endogenous opioid peptides: β-endorphin, prodynorphin, enkephalin, and orphanin/nociceptin encoded by *POMC*, *PDYN* genes (Kopinsky & Hyman, 2002).

Exogenous opioids (such as morphine and heroin) and endogenous opioid peptides (such as β-endorphin, enkephalin, and dynorphin) are thought to exert their pharmacological and physiological effects via binding to μ-, δ-, and κ-opioid receptors. Both central and peripheral nervous systems contain opioid receptors (Zhang et al., 2008). They are found in varying concentrations throughout the brain depending on their classification, but all receptors are highly abundant in the amygdala, nucleus accumbens (NAc), and caudate putamen (CP) (Zhang et al., 2008). These areas, along with the ventral tegmental area (VTA), are densely packed with gamma-aminobutyric acid (GABA)-ergic interneurons that form the intricate neural circuitry underlying opioid dependence (Kalivas, 2009; Kalivas & Volkow, 2005). The opioid system mediated by μ-, δ-, and κ-opioid receptors is implicated in the reward circuitry's dopaminergic activity. Numerous substances, including opioids, nicotine, alcohol, and stimulants, alter the opioid system significantly. In general, activation of the brain opioid system contributes to the reward effect and modulates the neurochemical and behavioral effects of various addictive substances (Lopez-Leon et al., 2021).
Numerous studies have established that these three opioid receptors are involved in the analgesic and addictive properties of opioid drugs. Among these three receptors, it has been suggested that the μ-receptor is the primary target for opioid addiction (Matthes et al., 1996). μ-receptor is the most receptive to morphine, and its stimulation results in pain relief and euphoria (Chiara & Alan North, 1992). It has also been found that the δ-receptor mediates antinociception at both the spinal and supraspinal levels (Heyman et al., 1988).

Although opiate addiction has reached epidemic proportions in recent years, opiates have remained relatively unstudied. Investigating the role of genetic variants in the etiology of addiction may improve treatment response and disease prevention. Identifying genes involved in neuroadaptation is being used in conjunction with genome-wide and candidate gene association studies to elucidate the genetic factor's underlying role in drug addiction. This further paves the way for a better understanding of what drives opioid addiction and specifically addresses it. This brief review summarizes recent research evidence on the genetic variants of opioid receptors associated with opioid addiction.

Genetic susceptibility to opioid addiction

Genome-wide association studies in opioid addiction

Genome-wide association studies (GWAS) examine genetic variants across multiple individuals' genomes to identify genotype-phenotype associations. GWAS have revolutionized the field of complex disease genetics over the last decade, revealing numerous compelling associations for human complex traits and diseases (Tam et al., 2019). GWAS has taken the lead surpassing the previous candidate gene-driven approaches. GWAS has paved the way for the identification of common genetic variants such as single nucleotide variants (SNVs), rare variants, and structural variants [e.g., copy number variants (CNVs)] (Gaddis et al., 2021).

Numerous large-scale GWAS of opioid addiction have been published in previous years (Berrettini, 2017). Opioids act primarily by activating μ-opioid receptors on GABAergic interneurons, inhibiting GABA release and thus disinhibiting mesolimbic dopamine neurons. Thereby, opioids increase dopamine levels in the nucleus accumbens (Johnson & North, 1992). In addition to previously identified genes involved in dopaminergic signaling (e.g., *ANKK1/DDRD2, DRD1,* and *DBH*) (Clarke et al., 2013; Garrido et al., 2011; Hoenicka et al., 2010; Perez de los Cobos et al., 2007), the specific variant encoding the μ-opioid receptor (*OPRM1*, rs1799971, A118G) has been extensively studied (Prom-Wormley et al., 2017).

Twin and family studies have estimated that additive genetic factors account for 50% of the risk of opioid dependence among closely related family members (Berrettini, 2017; Kendler, Prescott, et al., 2003; Tsuang, 2001).

Hancock et al., (2015) has discovered a common missense functional SNV (rs1799971) in the exon 1 of *OPRM1* gene (A encodes the wild-type asparagine allele, whereas G encodes the aspartate allele). Through a GWAS, an opioid addiction haplotype comprising of the C allele of rs3778150 and the A allele of rs1799971 described by Hancock et al., (2015) was associated with opioid addiction. This finding may support the hypothesis of the association of rs1799971 with opioid addictions (Schwantes-An et al., 2016; Haerian & Haerian, 2013). The most recent meta-analysis included three samples (8529 affected European American individuals and 71 200 opioid-exposed European American controls and 4032 affected African American individuals and 26029 opioid-exposed African American controls) totaling 82,707 Europeans confirmed that SNV rs1799971 was associated with opioid use disorder while there was no association among African Americans (Zhou et al., 2020). A significant association between variant rs1799971 and opioid/cocaine/heroin dependence was found in Asian populations, where no association was observed in African American or Caucasian people. Thus, the *OPRM1* rs1799971 variant may be a risk factor for Asians being vulnerable to an addiction to opioids or heroin (Haerian & Haerian, 2013).

In contrast to the above-described studies, Glatt et al., (2007) reported no significant role of rs1799971 in opioid addiction.

In addition to *OPRM1* gene SNVs, other genes related to the dopaminergic system have also been investigated. However, analyses of prodynorphin *(PDYN),* proenkephalin *(PENK),* and the κ *(OPRK1)* and δ-opioid receptors *(OPRD1)* have not consistently yielded reliable results with respect to opioid addiction (Zhang et al., 2008; Gerra et al., 2007; Franke et al., 1999; Mayer et al., 1997).

Previous research revealed that heterodimerization of the *OPRM1* and *OPRD1* genes can alter the opioid signaling pathway more than individual activation of the OPRM1 and OPRD1 receptors. More research revealed that heterodimerization of *OPRD1–OPRM1* resulted in physiological repercussions, necessitating further studies to determine how the coexistence of both receptors contributes to an increased risk of opioid addiction (Wu et al., 2021).

There are promising GWAS reports in opioid addiction that identify genome-wide significant risk alleles, but inconsistent results cast doubt on the association of these genes across ethnic groups. Larger samples with opioid addiction must be evaluated in different ethnic groups through candidate gene studies of opioid addiction.

Candidate gene association studies of opioid receptors

Numerous studies have established a link between opioid system gene variants and drug addiction-related phenotypes, but the findings are inconsistent. The inconsistency of genetic studies may be explained by multiple factors such as: inconsistency in phenotyping the variations, severity stage of diagnosis and diagnosis criteria of addiction, more studies with smaller sample size, insufficient statistics, ethnic heterogeneity, stratification strategy of population and wide phenotype range. The majority of studies used single nucleotide variations (SNVs) analysis, and several studies used hypothesis-based multi-SNP arrays that detect a significant proportion of common genetic variations (Orna Levran et al., 2012; Maher et al., 2011; Hodgkinson et al., 2008; Levran et al., 2008).

The μ, κ, and δ opioid receptors are all G protein-coupled receptors that work in conjunction with inhibitory G proteins and dopaminergic neurons to generate the physiological effects of opioids, according to one of the candidate gene studies (Chiara & Alan North, 1992).

OPRM1 gene

The opioid receptor gene 1 *(OPRM1)* is located on chromosome 6q25.2 (NCBI database, 2021c). Four exons comprise the primary subtype. The *OPRM1* gene, which encodes the opioid receptor, has been implicated in respiration, gastrointestinal motility, physical dependence, euphoria, and analgesia (Mistry et al., 2014). The μ- opioid receptor, *OPRM1* (G protein-coupled) is the primary site of action for endogenous opioids, opiate and opioid analgesics, and exogenous opioids such as methadone, heroin, and morphine (Orna Levran et al., 2012). β-endorphin binding to the μ- opioid receptor results in the disinhibition of dopaminergic neurons, which has been linked to reward and reinforcement and is thought to contribute to the development of drug dependence (Johnson & North, 1992). Numerous SNVs in this gene have been associated with opioid dependence and ethnic variation in opioid dependence (Baldacchino et al., 2019).

Various studies in diverse populations have demonstrated an association between the rs1799971 variant and opioid dependence and other substance dependencies. According to Bond et al., (1998) the most frequently occurring SNPs in the *OPRM1* gene are rs1799971 (A118G) and rs1799972 (C17T). The most studied *OPRM1* variant rs1799971 was shown to eliminate a potential N-glycosylation site in the extracellular domain, increase beta-endorphin binding affinity, and decrease receptor signaling efficacy (Bond et al., 1998). The rs1799971 variant of the *OPRM1* gene is prevalent among Europeans (15-30%) and Asians (40-60%). However, the variant is less prevalent among African Americans and Hispanics (1-3%) (Tan et al., 2003; Gelernter et al., 1999; Bergen et al., 1997). The variant rs1799971 was less prevalent among Africans (Kreek et al., 2005).

The rs1799971 minor allele variant was associated with opioid dependence in an Indian population (Kapur et al., 2007) and with heroin dependence among Sri Lankans (Dissabandara et al., 2021). The rs1799971 was associated with opioid addiction in Swedish (Bart et al., 2004), Chinese (Szeto et al., 2001), European Americans (Drakenberg et al., 2006) and Indian patients (Deb et al., 2010; Kapur et al., 2007; Tan et al., 2003) where some of the studies have contradicted this finding (Orna Levran et al., 2012; Glatt et al., 2007; Kapur et al., 2007; Tan et al., 2003; Shi et al., 2002).

The *OPRM1* gene variant, rs1799971, was not

associated with opiate addiction in a recent study in a Bulgarian population, which included 1842 opiate-addicted subjects (Bulgarians (18% allelic frequency of 118G) and Romas (Romani population) (20.2 percent allelic frequency of 118G)) and 1451 healthy volunteers (Nikolov et al., 2011). The study conducted by Kreek et al., (2005) showed a significant association between the rs1799971 variants, but in contrast, no association was seen between the rs1799972 variant and opioid dependence.

Due to the contrasting findings regarding the association of the rs1799971 SNV with opioid dependence across different ethnic groups, it is critical to further investigate this variant in multiethnic cohort genetic association studies.

OPRD1 gene

The opioid receptor delta 1 *(OPRD1)* gene encodes for the δ-opioid receptor is located on chromosome 1p35.3 (NCBI database, 2021a). Numerous studies have established a link between variations in the allelic frequencies of *OPRD1* SNVs and opioid addiction. There are hundreds of variants of the *OPRD1* gene that have been studied with association to opioid addiction, but only a few variants have been studied with respect to different ethnic groups (Baldacchino et al., 2019).

Hunag et al. investigated the rs2234918 SNV among the Han Chinese and discovered that the minor C allele of rs2234918 in *OPRD1* is considered a risk allele for heroin dependence (Huang et al., 2019). While Mayer et al. also reported that rs2234918 SNV of the *OPRD1* gene is associated with opioid addiction among the German Caucasian heroin addicts (Mayer et al., 1997). In contrast to the above studies, Zhang et al., (2008) (1063 European Americans: 620 cases of alcohol, cocaine, and opioid dependence and 443 control subjects) reported that there were no associations between the silent mutation rs2234918 with opioid addiction among the European Americans in a candidate gene association study.

In addition to the SNV mentioned above, Zhang et al., (2008) also studied several SNVs among the European Americans, including eleven *OPRD1* variants and seven *OPRK1* variants. Among the studied variants, rs1042114 was the only variant detected in exon 1 of the *OPRD1* gene, demonstrating a significantly increased frequency of the minor G allele in opioid-dependent subjects compared to the controls. Similarly, Crist et al., (2013) also found that rs1042114 was significantly associated with opioid addiction among European Americans and African Americans. Based on the results, both the rs1042114 (G80T) and rs2234918 (T921C) in the *OPRD1* gene are risk factors for opioid drug addiction among mixed ethnic groups (Crist et al., 2013; Zhang et al., 2008).

Levran et al., (2008) conducted a study among the Americans and Israel people and found that rs2236861, rs2236857, and rs3766951 of the *OPRD1* gene were suggestively associated with heroin addiction. Similarly, Nelson et al., (2014) also concluded that rs2236861 and rs3766951 were considered risk factors for opioid addiction but did not observe any significant association for the common rs2236857 SNV for opioid addiction. A few *OPRK1* gene variants have been associated with SUDs; however, most are silent and do not affect gene expression (Mayer & Höllt, 2006).

OPRK1 gene

The opioid receptor kappa 1 *(OPRK1)* gene is located on chromosome 8q11.23 (NCBI database, 2021b). Earlier, the studies reported that the *OPRK1* gene has been playing a role as an anti-addictive effect and produces dysphoria, but recent evidence suggests that prolonged exposure to drugs activate the *OPRK1* gene, which may play a key role in motivational aspects of dependence through modulation of basal and drug-induced dopaminergic tone (Wee & Koob, 2010; Kreek et al., 2002).

Despite numerous studies examining the role of the κ opioid receptor, the evidence for a link between *OPRK1* SNPs and opioid dependency is inconsistent, with results considerably varying

between ethnic groups (Mistry et al., 2014).

Gerra et al., (2007) genotyped 106 heroin - dependent subjects and 70 healthy controls for rs1051660 variant in the *OPRK1* gene among Western Europeans. The study concluded that the rs1051660 variant is more prevalent among heroin addicts. Meanwhile, Yuferov et al., (2004) also concluded that the rs1051660 variant is a risk factor for opioid use disorders. Mistry et al., (2014) genotyped 202 healthy individuals and 202 opium addicts with rs997917, rs6985606, and rs6473797 variants of the *OPRK1* gene and found these variants reported significant association with susceptibility to opioid dependence among Iranians. In contrast to these studies, Zhang et al., (2008) reported that rs997917 SNV was not associated with opioid addiction.

Table 1: Summary of selected opioid receptor genes

Gene	Receptor	Reward pathway effect	Variants	Location	Findings	References
OPRM1	μ opioid receptor	euphoria and respiratory depression	rs1799971	Exon 1	rs1799971 variant has been reported as a risk factor for opioid addiction among Europeans, Indian, Caucasians, Malaysian, Chinese.	(Schwantes-An et al., 2016)
			rs1799972	Exon 1	rs1799972 (T) allele is less consistent across ethnic groups and is not widely linked to opioid dependence despite being widely studied.	(Brattwall et al., 2010)
OPRD1	δ opioid receptor	anxiolysis	rs2234918	Exon 3	The *OPRD1* exon III rs2234918 is associated with opioid addiction among Han Chinese, Caucasians.	(Huang et al., 2019; Compton & Volkow, 2006; Mayer et al., 1997)
			rs1042114	Exon 1		
			rs2236861	Intron 1	*ORPD1* SNP, rs2236861, was associated with non-dependent opioid use	(Nelson et al., 2014; Levran et al., 2008)
			rs2236857	Intron 1	Associated with OUD in individuals of European descent	
			rs3766951	Intron 1	Associated with opioid addiction risk among Caucasian ancestry	
OPRK1	κ opioid receptor	dysphoria	rs1051660	Exon 2	Associated with opioid addiction as a risk factor among African American, Caucasian, Hispanic, Asian-American, and mixed ethnic groups.	(Gerra et al., 2007; Yuferov et al., 2004)
			rs997917	Intron 2	Associated among Iranian but not associated among Americans and Europeans.	(Mistry et al., 2014; Zhang et al., 2008)

Summary

The review aimed to summarize the evidence for opioid receptor genes associated with opioid addiction. μ, κ, and δ opioid receptor subunits are encoded by the *OPRM1*, *OPRD1*, and *OPRK1* genes, respectively. The majority of the studies included in this review were retrospective genome-wide association studies in which opioid addiction-related genotypes and SNVs were investigated.

Previous studies included African Americans, Han Chinese, Hispanics, Europeans, and Caucasians as common ethnic groups with significant ethnic variations in the association between genetic variants and opioid dependence (Baldacchino et al., 2019). There were several larger studies which had been studied among varying ethnic groups, including the Zhang et al., (2008) study, which included 1063 European Americans, Nagoya et al., (2018) included 1002 Malay males, and Nelson et al., (2014) included 2954 Australians. Although compelling results have been obtained from the genetic studies of opioid addiction, it is necessary to investigate the association of opioid receptor gene variants among varying ethnic groups, both with small scale and large-scale studies, to find the genetic susceptibility. A detailed analysis of the effects of these genes on the pathophysiology and metabolism of opioids will provide additional insight into the aetiology of such disorders.

The *OPRM1*, *OPRD1*, and *OPRM1* genes were all implicated in the development of opioid addiction. These genes encode receptors and signaling molecules involved in the pathophysiology of substance use disorders (Mistry et al., 2014). Despite a high genetic predisposition to opioid dependence, environmental factors play a significant role in this opioid use disorder, as they do in many other multifactorial diseases. Hence, studies should focus on interactions between genes and the environment, or epigenetics, to understand more about opioid and other substance use disorders.

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Review Article

Host Genetic Susceptibility and Impacts of Dietary Factors on Covid-19

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Abstract

COVID-19, a disease caused by SARS coronavirus 2 (SARS-CoV-2) has been a virus which is causing a global issue due to the fact that it resulted in a pandemic. The SARS coronavirus 2 binds with the angiotensin-converting enzyme 2 (ACE-2) receptors expressed in various human organs including the lungs. The SARC-CoV-2 causes respiratory disease ranging from mild to life-threatening pneumonia. Covid-19 is much feared due to its ultimate outcome such as multi-organ damage as a result of the production of a high level of pro-inflammatory cytokines referred to as “cytokine storm and resulting increased mortality and morbidity. Outcomes of the COVID 19 infection are associated with several factors that include demographic, environmental, behavioural, dietary, and genetic factors. In addition, male gender, ethnicity, older age, obesity, and several chronic diseases affect the severity of the disease. Genetic susceptibility and dietary factors involved with COVID – 19 infectivity are current areas that are widely researched and likely to play a major role. Through Genome Wide Association Studies (GWAS) and other studies done worldwide, scientists have discovered variants related to the susceptibility and severity of COVID-19. Among published reports, the involvement of ACE-2, TMPRSS2, 3p21.31 gene cluster, OAS1, OAS2 and OAS3, TYK2, DPP9, IFNAR2, CCR2 TMEM189–UBE2V1 genes, IFNL4, TLR7, LZTFL1, CCHCR1, CCNG1, ACSF3, FPR1, NKG2C/HLA-E, NLRP3, DNAH7 and CLUAP1, DES and SPEG, STXBP5, TOMM7, WSB1, PIEZO1, SCN5A, APOL1, PRKRA, GC, NADSYN1, VDR, and CYP2R1, PPCDC, VDR, DMGDH is summarized in this review. Similarly, this review concluded the role of nutrition in terms of the importance of a balanced diet and functional foods, micronutrients, probiotics, and how the omega 3/omega 6 ratio helps to prevent the severity of COVID-19.

Key words: COVID 19, Genetic susceptibility, Nutrition, SARS-CoV-2

Introduction

Since the onset of the covid pandemic, scientists have been investigating the reasons behind the variability of Covid 19 symptoms. Some individuals manifest severe life-threatening symptoms while others only have mild or negligible symptoms. Studies across the globe currently is in an attempt to shed light on the susceptibility factors of the host associated with the severity (Asgari and Pousaz, 2021).

The COVID-19 symptoms can be described in a spectrum, categorized in to asymptomatic, mild to moderate, severe, and critical disease. Fever, cough, headache, fatigue, and breathing difficulties are among the most common symptoms. It has been reported that nearly a one third of the individuals are asymptomatic. Among the symptomatic population, nearly 80% has been reported as having mild or moderate symptoms while the remainder manifests severe symptoms including severe breathing difficulty, and lung damage where approximately 5% out of the latter develops respiratory failure, ARDS, shock or multiple organ failure leading to death (Velavan et al., 2021).

The association of demographic factors, environmental factors, behavioural factors, other diseases, dietary patterns, and genetic factors with Covid – 19 symptoms have been described by several studies previously. Advanced age, male sex, obesity and other non-communicable diseases have been identified to be related to increased severity of the disease (Grivas et al., 2021). Ethnicity also has been identified as a probable factor associated with the severity of Covid- 19, which has paved the path to investigate genetic variants across the human genome in Covid – 19 patients in different ethnicities and different groups of people (Hu et al., 2021). Diet plays a huge role in human life where health and diet have been described under many contexts. Researchers have also investigated the effect of dietary patterns on the severity of Covid – 19 (Salazar-Robles et al., 2021).

Therefore, this review, summarizes the association of genetic factors and dietary factors with the increased susceptibility and severity of Covid-19.

Host Genetic susceptibility for Covid – 19

Worldwide scientists are interested in the phenomenon of individual inter variability when it comes to the severity of infectious diseases, where attention is invariably has been towards investigating the genetic factors through Genome Wide Association Studies (GWAS). It has been challenging to identify the impact of genetic variants on infectious diseases due to the other major influential factors such as health care access and socio-demographic factors. Scientists all over the world have collaborated to form large definitive study populations related to Covid-19 to investigate the genetic impact and the severity of Covid-19 symptoms (Asgari and Pousaz, 2021).

Similarly, another GWAS study conducted with 1980 Covid-19 patients, has confirmed the presence of the 3p21.31 gene cluster comprising signals from 6 genes (i.e: SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6, and XCR1) as a locus for genetic susceptibility in patients infected by Covid-19 who were suffering from respiratory failure. This locus also showed a probable connection to the ABO blood grouping system with the detection of an association signal at locus 9q34.2 (rs657152) responsible for ABO grouping system (Severe Covid-19 GWAS Group et al., 2020). The study has detected the variant rs11385942 (insertion–deletion GA or G variant) as a potential risk variant. The GA allele is associated with the reduced expression of CXCR6 and increased expression of SLC6A20, and LZTFL1 which are connected to lung cells (Severe Covid-19 GWAS Group et al., 2020). The GA allele of the variant rs11385942 further has been confirmed as a risk allele as it has been identified in higher frequency from patients under mechanical ventilation compared to those who only require oxygen supplementation (Severe Covid-19 GWAS Group et al., 2020). The study further has identified the blood group “A” having a higher risk than other blood groups and a

probable protection expressed in blood group "O" compared to other blood groups through the association signals between rs11385942 and rs657152 (Severe Covid-19 GWAS Group et al, 2020). An interesting discovery following the findings of Severe Covid-19 GWAS Group et al, 2020 was that the region of risk loci is inherited from Neanderthals and is seen in almost half the South Asian populations and around 16% in Europe (Zeberg and Pääbo, 2020).

Another genome wide study including 2,244 severely ill COVID-19 patients has reported novel significant associations on chromosome 12q24.13 (rs10735079) in a gene cluster (OAS1, OAS2 and OAS3) encoding activators of antiviral restriction enzyme; on chromosome 19p13.2 (rs74956615) related to the gene encoding tyrosine kinase 2 (TYK2); on chromosome 19p13.3 (rs2109069) encoding dipeptidyl peptidase 9 (DPP9); and on chromosome 21q22.1 (rs2236757) related to interferon receptor gene IFNAR2. This study has also revealed that the monocyte–macrophage chemotactic receptor CCR2 is also associated with severe COVID-19 (Pairo-Castineira et al., 2020).

IL-1 is found to be elevated in COVID-19 patients especially critical patients with severe symptoms who suffer from the cytokine storm (Wang et al., 2020). GWAS conducted among 332 COVID-19 patients in Hong Kong has identified a significant gene variant (rs6020298) located in IL-1 signalling pathway that enhances the level of IL-1 which aggravates the severity of COVID-19 in a read-through transcript TMEM189–UBE2V1 in the 20q13.13 region that is found to be connected with the innate immune signalling. Further the blockage of IL-1 in critical patients of COVID-19 in one of the clinical studies have shown that the respiratory function to be improved in 72% of the patients (Wang et al., 2020).

LZTFL1 gene variant rs73064425 has a possible action in impairment of respiratory epithelial cell function (Rescenko et al., 2021) where this has been identified in increased frequency from critical COVID-19 patients (Horowitz et al., 2020) and may indicate a probable impact on increasing the severity of COVID-19 symptoms. CCHCR1 produces a protein that is involved in cytoskeletal remodelling and mRNA turnover, where the variant rs143334143 of CCHCR1 gene has been identified to impair this function. Further this variant (rs143334143) has been identified in high frequency in critically ill patients of COVID-19 (Horowitz et al, 2020). Variants of DPP9 that enhances the dipeptidyl peptidase secretion may have a possible influence on COVID-19 severity, where increased expression of the variant rs2277732 of DPP9 in severe covid-19 patients was detected (Horowitz et al., 2020). Further 3 novel variants; CCNG1 (rs79833209), ACSF3 (rs4782327), FPR1 (rs12461764) were discovered as common genetic variants that affect the COVID-19 susceptibility and severity by another GWAS (Horowitz et al., 2020). The mechanisms by which these affect the severity has not been clearly explained, thus further investigations must be carried out in this avenue.

According to the UK biobank data, 8 potential genetic risk loci associated with mortality of COVID-19 has been identified. The 8 super variants have been identified in a consistent manner across populations with respect to Covid-19 related deaths. Genes DNAH7 and CLUAP1 (cilia dysfunction), DES and SPEG (cardiovascular disease), STXBP5 (thromboembolic disease), TOMM7 (mitochondrial dysfunctions), WSB1 (innate immune system), and DNAH7 (newly identified gene in bronchial epithelial cells, downregulated in SARS-CoV-2 patients) (Hu et al., 2021). Another study conducted using UK biobank data has discovered a connection between the gene PIEZO1 and COVID-19 mortality. PIEZO1 is a gene that moderates the epithelial cell response to blood flow by encoding proteins which make a cation channel as well as may have a role in pulmonary inflammation (Cheng et al., 2020). The exact mechanism by which this gene is associated to COVID-19 severity is still under investigation. The same study also has stated the susceptibility of ethnic groups with respect to the variants of the gene PIEZO1 (Cheng et al., 2020).

Interferons play a vital role in host antiviral signalling and stimulate release of crucial components of the early host response to viral infection. Interferons (IFNs) are considered a crucial molecule for the COVID-19 disease outcomes as well. A case (177 patients) and control (445 healthy individuals) study revealed that IFNL4 gene variants (affects production of interferons) may be predisposing to the outcomes of COVID-19 (Saponi-Cortes et al., 2021). IFNAR2- rs13050728 which is a loss of function mutation in the type-1 interferon receptor also increases the severity of the disease (Horowitz et al, 2020). Elevated expression of the interferon receptor subunit IFNAR2 with respect to the studies conducted in order to discover the beneficial role for type I interferons has shown reduced risk for severe form of COVID-19 (Pairo-Castineira et al., 2021). Another study has also identified the probable connection of IFNAR2 gene to COVID – 19 susceptibilities as well as prognosis (Liu et al., 2020). A whole genome sequencing study using 4 cases of young men severely affected by Covid-19 (two unrelated families) discovered a noteworthy loss-of-function variants in TLR7 gene that stimulate IFN-ɤ which is located in X-chromosome (Van der Made et al., 2020).

A study finding has concluded that variants in NKG2C/HLA-E axis (KLRC2del and HLA-E*0101) to have an impact on severity of SARS-CoV-2 infection, thereby thought to be useful as a potential predictor of high-risk for severe COVID-19 (Vietzen et al., 2021).

NLRP3 gene has been identified as a main player of the innate immune system which mediates the organization of host-immune response. Single nucleotide variants in the NLPR3 gene (rs10754558 C>G and rs10157379 T>C) were found to be positively associated with SARS-CoV-2 infectious symptoms, in a study conducted using 308 critically ill COVID-19 patients. The study also concluded that rs10754558 C>G of the NLPR3 gene to be a significant predictor of the risk of COVID – 19 susceptibility and severity (Maes et al., 2021).

The genetic susceptibility for COVID-19 associated cardiac involvement has been described by a group of researchers where a variant of SCN5A gene (p. Ser1103-Tyr) has been identified in a cohort of diseases affected African males (Giudicessi et al., 2021). APOL1 gene has also gained attention with respect to Kidney involvement of COVID-19 disease in individuals with African ancestry (Friedman, 2021).

ACE-2 (Angiotensin-2 Conversion Enzyme) has been found as a mediating protein for SARS-CoV-2 to access the human host cells. SARS-CoV-2 gains entry to host type II lung cells by binding ACE2. SARS-CoV-2 binding to ACE2 is facilitated by transmembrane protease, serine 2 (TMPRSS2), which cleaves one of the surface proteins of the virus. Therefore, variations that will result in upregulation of both these ACE 2 and TMPRSS2 in host cells increase the SARS-CoV-2 infectivity. Both these enzymes are regulated by Androgen Receptor gene explaining the increased susceptibility of male gender to the severe form of the disease (McCoy et al., 2020) (Strope et al., 2020) (Gibson et al., 2020). Scientists also have predicted that the gene variants that are associated with diseases such as androgenetic alopecia, prostate cancer, benign prostatic hyperplasia, and polycystic ovary syndrome can also be associated with increased risk of COVID-19 susceptibility. However, further studies are necessary for the establishment of these connections (Wambier et al., 2020) (McCoy et al., 2020). Further ongoing studies investigate the length of CAG repeats in the Androgen Receptor gene in COVID-19 patients (McCoy et al., 2020). An Italian group of researchers also have discovered the relation of TMPRSS2 gene variant as a potential predictor of risk in male patients (Asselta et al., 2020). Singh et al., (2021) also describes the association of ACE2 and TMPRSS2 variants withthe severity or protection from COVID-19 in different populations (Singh et al., 2021).

Another study has discovered the variants in TMPRSS2 gene (c.331G>A, c.23G>T, and c.589G>A) connected to the viral entry to human cells (Latini et al., 2020). Apart from ACE-2 and

TMPRSS2 there are other proteins that are involved with the entry of this virus. A study conducted using 131 Covid-19 patients have discovered a missense variant in PCSK3 gene (c.893G>A) (Latini et al., 2020) connected with the severity. A cohort study (35 COVID -19 patients) showed that variants in a protein kinase enzyme (activated by viral RNA); protein kinase activator A, also known as PRKRA, to be having an impact on the immune response mediated by IFN (Benetti et al., 2020)

Proper nutrition with vitamins and minerals is essential for an efficient immune system. Vitamin D, Zinc and Selenium are some of the nutrients that are responsible for a healthy immune system and have been discovered to be important in prevention and recovery from COVID-19 infection. Vitamin D has further been identified as an important nutrient neutralizing the SARS-CoV-2 impact through the viral binding to the VDR receptor supporting in the control of immune response. A study conducted using a population of COVID- 19 infected patients in UAE has discovered 12 genetic variants in Vitamin D related genes GC, NADSYN1, VDR, and CYP2R1 (Al-Anouti et al., 2021). Variants related to vitamin D status, Zinc and Selenium levels have been found in increased frequency in a study conducted using 120 Serbian individuals (Adult and paediatric) infected with Covid- 19 (DHCR7/NADSYN1 rs12785878, GC rs2282679, CYP2R1 rs10741657, and VDR rs2228570 variants related to Vitamin D; PPCDC rs2120019 variant related to Zinc and DMGDH rs17823744 related to selenium) (Kotur et al., 2021).

Genomic studies to understand the influential factors of the host for COVID – 19 is a rapidly expanding research field as well as it can be of high importance in the aspects of understanding the multiorgan involvement, virulent factors of the virus, risk prediction, prevention, treatment, and management of COVID – 19. This knowledge can be used for downstream processes such as developing drugs or vaccines for the current and novel variants of the SARS-CoV-2.

Role of nutrition in Covid 19 susceptibility and severity.

Nutrition/diet is one of the important environmental factors that interact with our genetic makeup to maintain normal development and homeostasis. A balanced diet provides all essential nutrients required to the body and it helps to maintain a healthy body status by reducing the risk of chronic non-communicable diseases including type 2 diabetes mellitus, cardiovascular diseases, and cancer (Simopoulos et al., 2021). A poor diet is considered as one of the leading factors for death and according to the statistics where one in five global deaths are associated with poor diet (Afshin et al., 2019).

Most people infected with SARS-CoV-2 showed no symptoms or mild symptoms which can further progress into a life-threatening condition due to several factors. People who are suffering from severe pulmonary diseases, chronic non-communicable diseases, and immunocompromised patients are more vulnerable to Covid-19 infection. In addition, obesity is considered as one of the main risk factors that cause the severity of the disease (Skrajnowska et al., 2021). People with an increased BMI have a poor prognosis of the disease due to the high pressure in the pleural cavity and reduced lung recruitment capacity which in turn causes atelectasis and alveolar collapse (Hibbert, Rice and Malhotra, 2012). Other than that, adipose tissue in obese people produces a high level of pro-inflammatory cytokines which in turn exacerbate the inflammatory conditions in people with COVID-19 and lead to acute respiratory distress syndrome or multiple organ failure (Makki Froguel and Wolowczuk, 2013). Similarly, cardiovascular diseases and diabetes have been discovered as two other coinciding diseases with COVID-19. People with diabetes have a high potential of getting the severe form of COVID-19 due to the excessive secretion of cytokines, interleukin-6, and C-reactive protein which exacerbates the inflammatory states (Tsalamandris et al., 2019). Therefore, it is necessary to find a way to reduce the cytokines storm in high-risk

groups of COVID-19. Diet plays an important role influencing the levels of gene expression of cytokines and thus modulate inflammation and oxidative stress associated with Covid 19 infection (Lange,2021). People who are infected with COVID 19 have dysregulated immune response by increased secretion of cytokines and attenuated interferon response to the pathogenic virus. This status is further enhanced by conditions of obesity and diabetes mellitus by decreasing the interferon response without infections (Santos et al., 2021).

Although there are several factors associated with the pathology of Covid-19, insulin resistance is considered as one of the main risk factors that cause impairment of metabolic functions (Govender et al., 2021). Therefore, people who are suffering from the disorders of insulin resistance such as diabetes and obesity should consume a low carbohydrate diet as it provides a safe and effective way to maintain glycaemic control and evade insulin resistance (Skrajnowska et al., 2021).

In addition, nutritional status plays a key role as a driver of SARS-CoV-2 virulence. It has been identified that malnutrition has a direct impact on Covid 19 progression as it is the primary cause of immunodeficiency worldwide (James et al., 2021). An inadequate diet and the resulting malnutrition impair the immune response in Covid 19 patients which in turn increase the mortality rate due to poor fighting capacity against the disease (Mertens and Penalvo, 2021). In this context, some nutrients are already highlighted for their role in improving the immune response thereby lowering the susceptibility for COVID – 19 infection (Octavia and Harlan, 2021).

Long-chain polyunsaturated fatty acids including omega-3 and omega-6 are mainly involved with the regulation of inflammation (DiNicolantonio and O'Keefe, 2018). Hence, the deficiency can lead to an increase in the susceptibility to viral diseases including novel coronavirus. Omega-6 fatty acid acts as a substrate to produce pro-inflammatory prostaglandins and leukotrienes which help to enhance the immune response over invasion by pathogens (Innes and Calder, 2018). Whereas omega-3 fatty acids suppress the immune responses by inhibiting the activation of neutrophils and monocytes. As omega-6 and omega-3 fatty acids and their metabolites have opposing properties, a balanced ratio is important to maintain health. Therefore, a diet rich in omega 6 and low in omega 3 should be consumed to enhance the immune responses against inflammation. It is recommended to use a mixture of fish fatty acids including docosahexaenoic acid (DHA) + eicosapentaenoic acid (EPA) to reduce oxidative stress and the symptoms of acute respiratory distress in Covid 19 (Skrajnowska et al., 2021).

Micronutrients also play a major role in supporting the immune system. Vitamin B group plays a vital role in the amino acid synthesis, tissue formation, and the regulation of gene expression. Among the Vitamin B group, Vitamin B6 (pyridoxal 50-phosphate) regulates the immune function as a co-factor in generating metabolites with immunomodulating effects. Therefore, the deficiency will ultimately lead to the deterioration of protein synthesis and tissue formation (Shakoor et al., 2021). Similarly, Vitamin C helps to boost the immune system by synthesizing the antioxidant in the skin which in turn enhances the barrier function against pathogens. Further, Vitamin C is associated with the maintenance of immunity by accumulating in phagocytic cells to enhance chemotaxis, phagocytosis, generation of reactive oxygen species, and ultimately microbial killing (Carr and Maggini, 2017). Due to the antioxidant and free radical scavenging activities of Vitamin C, it has the ability to fight against COVID 19. Clinical trials have confirmed that the ability of Vitamin C to reduce the frequency, duration, and severity of the common cold and the incidence of pneumonia. Most importantly, it has proved that the high-dose of intravenous vitamin C may help to reduce cytokine storm in severe SARS-CoV-2 infection. Therefore, the consumption of Vitamin C-rich foods is important to maintain the daily requirement to reduce the predisposition to COVID 19 infection (De Melo and Homem-de-Mello,2020).

Vitamin D also reduces the infection by modulating the formation of antimicrobial proteins including defensins, and cathelicidins in the skin. In addition, Vitamin D receptors present in immune cells are responsible for modulating the responses to viral lung diseases and protecting against infectious respiratory diseases in the prevention of COVID-19 (Octavia and Harlan, 2021). Further studies reported that vitamin D deficiency as a risk factor for COVID-19 infection (Jude et al., 2021). Further, Whittemore (2020) has identified that the rates of COVID 19 infection are higher in countries at higher latitudes due to the low exposure to sunlight. Hence, people should mainly focus on a diet rich in Vitamin D to reduce the infection of COVID 19. Among the different types of food, fatty fish and egg are considered as good sources of Vitamin D (Whittemore,2020).

Further, zinc and selenium play an important role in the regulation of immune response. Selenium can protect the immune system mainly from viral infections by increasing the production of interleukin-2 (IL-2) which exhibits immunomodulatory effects (Khatiwada and Subedi 2021). Therefore, selenium can improve the immunomodulatory effects in patients infected with SARS-Cov-2. Similarly, zinc can also regulate the inflammatory responses by activation, proliferation, and maturation of the immune cells such as leukocytes and lymphocytes. In addition, zinc can prevent the SARS-Cov-2 from entering cells. Hence, adequate levels of zinc and selenium are important for immune function and viral clearance of SARS-CoV-2 infection (Nikola et al., 2021).

Polyphenols, flavonoids, and carotenoids are bioactive compounds that exhibit antiviral properties. Polyphenols are naturally found in fruits and vegetables other than cereals, dry legumes, chocolate, and beverages, such as tea and coffee which exhibit potent antioxidant and anti-inflammatory effects. Polyphenols have been identified as the potential bioactive compound that can be used to prevent respiratory viral infections including COVID 19 (Paraiso, Revel and, Stevens, 2020). Polyphenols can block the angiotensin-converting enzyme 2 (ACE-2) receptors that have been identified as the functional SARS-CoV-2 receptors to prevent the entry of the virus and its replication in the host cells (Lange, 2021). Similarly, flavonoids also exhibit antiviral and immunomodulatory effects, thereby reducing the risk of infection with SARS-CoV-2 by inhibiting various inflammatory cytokines. Flavonoids can also bind with ACE-2 receptors and block the entry of the SARS-CoV-2 virus (Alzaabi et al., 2021). In addition, carotenoids are a group of natural pigments that can act against inflammation and oxidative damage. Therefore, diets rich in carotenoids can be utilized in the treatment of the acute phase of COVID-19 by targeting the inflammatory storm resulting from viral infections (Khalil et al., 2021). Therefore, it is recommended to consume at least five portions of fruit and vegetables every day to gain the advantages of the above-mentioned bioactive compounds in the prevention of the severe form of COVID-19 infection.

Even though, it is commonly known that SARS-CoV-2 mainly affects the respiratory system, it can cause gastrointestinal infections as well. Intestinal endothelium contains receptors for the SARS-CoV-2 virus and the number of ACE-2 receptors increases with age by providing a potential entry mechanism for the SARS-CoV-2 virus. The entry of the SARS-CoV-2 virus can lead to imbalance of commensal gut microbiota (intestinal dysbiosis) that cause gastrointestinal infections. Therefore, probiotics provide some beneficial effects on intestinal dysbiosis by increasing the commensal bacteria including Lactobacillus and Bifidobacterium in people with COVID-19 infection a (Kurian et al., 2021).
In addition, natural functional compounds are identified as potential agents that exhibit antiviral and immunomodulatory effects. For example, vitamin C in fruits such as pomegranate and elderberry, allicin in garlic and onion, piperine in black pepper, gingerols in ginger, curcumin in turmeric, kaempferol, and methylglyoxal in honey are considered as health-promoting food ingredients to mitigate COVID-19 symptoms, however, the validated scientific evidence for the efficacy of these foods is not available

(Skrajnowska et al., 2021).

The importance of a healthy diet in prevention and management of COVID-19 and its consequences have been discussed in various studies conducted across the world. Among them, many studies have confirmed the necessity of a well-balanced diet to enhance the immune response against Covid-19 viral infection. It has also been pointed out that a healthy diet rich in vitamins, minerals, and balanced ratio of omega 6 and omega 3 help to mitigate the severity of the condition. Therefore, it is important to consume nutrients according to the RDA (recommended dietary allowance) requirement to enhance the optimal function of the immune system and mitigate Covid-19 infection and outcomes.

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Original Article

Aerva lanata; A cure or a cause for kidney diseases; A brief overview

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Abstract

At present the phyto-preparations are widely used in clinical practice and among them *Aerva lanata (A. lanata)* is commonly known as "Polpala" in Sinhalese, which is prescribed to alleviate kidney diseases in Complementary and Ayurvedic Medicine (CAM). The infusion of the plant extract has resolved urolithiasis and has induced diuresis in patients relying on CAM therapy. Scientific data supports this traditional claim. However, a controversy exists that the prolong use of the plant material may cause renal damage. Scientific literature also does not put forth the negative effects of using a long-term basis, though the dried plant preparations are readily available in the form of herbal tea in many countries. Therefore, this overview is a compilation of both the beneficial and detrimental effects of the use of plant material as a treatment in kidney diseases. Evaluation of the current literature supports the belief of nephrotoxicity in long term administration over its reported nephroprotection. Thus, the author reports that people who tend to self-medicate with *A. lanata* should be vigilant due to its possible adverse effects.

Key words: *Aerva lanata*, "Pashanabeda", Herbal diuretics, Anti urolithiatic herbs, Nephrotoxicity

Introduction

Aerva lanata is commonly known as " Polpala" in Sinhalese is a medicinal plant that belongs to the family Amaranthaceae (Figure 1). The plant is mostly adapted to grow in the drier parts of the tropics and the sub tropics of the world (Goyal et al., 2011). It is very much popular among natives of South Asian countries specially in India and Sri Lanka due to its versatile medicinal value. In Sri Lanka the plant has shown to be effective as an anti-inflammatory, anti-helminthic and anti-bacterial remedy (Gunatilake et al., 2012). Further it has been documented as a medicinal plant with mild-analgesic properties (Gunatilake et al., 2012). In Indian folk medicine *A. lanata* has shown to be effective in the treatment of diabetes mellitus, haematemesis, bronchitis, nasal bleeding, cough, scorpion stings and spermatorrhoea (Chowdhury et al., 2002).

Figure 1: Aerial parts of *A. lanata*

Among all its other medicinal uses, *A. lanata* is a trusted medication to alleviate the disease conditions associated with the urinary system in the countries where Complementary and Ayurvedic medicine (CAM) is in practice. Therefore, *A. lanata* is considered as a credible herb to restore the normal kidney function. Scientific evidences claim that the plant is shown to be effective in acute renal injury caused by nephrotoxins such as cisplatin and gentamicin (Shirwaikar et al., 2004). Furthermore, the plant has demonstrated anti urolithiatic and diuretic properties (Selvam et al., 2001; Soundararajan et al, 2006; Arthi et al., 2012).

"Pashanabeda" is a Sanskrit term that is cited to identify the group of plants extensively used in the Ayurvedic system of medicine, in order to dissolve the urinary calculi and stones (Gajalakshmi et al., 2012). Among herbal remedies with anti-urolthiatic property *A. lanata* is ranked at a top level in the group of "Pashanabeda". Allopathic medications and surgical interventions are available to combat the development of stones (Kumar and Clark, 2016).

However, these methods mostly failed to address the urolithiatic pathophysiology and therefore the underlying cause of calculi remains silent resulting in the repeated episodes of stones (Pareta et al., 2011). On account on the better safety, cultural acceptability and lesser side effects over the conventional counterparts, people tend to use *A. lanata* to attenuate kidney problems since antiquity (Jayaweera, 1981). Perhaps, this strong belief has led people to even self- medicate with dry or fresh plant material as a herbal drink or porridge according to their own interest (Priyashantha and Mahendranathan, 2020). In fact, the dried plant material is often sold by street vendors in the dry zone of Sri Lanka and across the country, furthermore, the dry matter is available in Ayurvedic outlets and pharmacies in a form of 'ready to use' sachet packets. Though *A. lanata* has shown to be credible in restoring the deteriorated kidney function in Sri Lankan Ayurvedic medicine, infusion of plant extract is not recommended over a long period due to the belief that it can have adverse effects on the urinary system (personnel communication).

Hence, in this review the author attempted to highlight the potential harmful effects of *A. lanata* over its documented beneficial effects on the urinary system. It is believed that the information presented may raise the awareness among the scientific community, perhaps to protect the public from the boundless use of *A. lanata* and other herbal remedies as a treatment for kidney diseases.

Search Methodology

In the first phase of this study, a bibliographic investigation was accomplished by analyzing worldwide scientific databases (PubMed, Scopus and Google Scholar) for the available information on clinical applications or uses of the plant. The plant was browsed along the key words *"Aerva lanata"*, "medicinal uses", "diuretic activity", "anti-urolithiatic" and "nephro toxicity". Available literature was screened and articles relevant to nephr-oprotection and nephrotoxicity were thoroughly scrutinized to collate the scientific evidences supporting the proposed title of the article. The plant is not extensively studied on this regard and therefore the available literature was minimal, to validate safety and efficacy on the urinary system. However, most of the literature cited that the plant possesses beneficiary effects on the urinary system. Nevertheless, few documentary evidences supported the belief of the Ayurvedic physicians of Sri Lanka claiming that the long-term use of the plant may produce harmful effects on kidney.

Beneficial effects of *A. lanata* on the urinary system

3.1. Anti - urolithiatic properties

The mechanism of stone formation proceeds through urinary saturation followed by super-saturation with stone forming constituents resulting in crystal nucleation and aggregation leading to crystal retention by the urinary epithelium over which the calculi progresses. Phyto-pharmaceutical agents capable of impeding the nucleation phase will interfere with further stages of stone formation thereby preventing lithiasis. *A. lanata* has demonstrated its ability to prevent crystal aggregation, growth and sedimentation, thereby combating urinary calculi (Alok et al., 2013).

3.1.1. In - vitro studies

In- vitro anti urolithiatic property of the flavonoid and phenol rich fractions of *A. lanata* was evaluated by Mandal et al., in 2017 and 2019. The investigators studied the effect of *A. lanata* on aggregation of stone forming components. Calcium oxalate crystals obtained were used for the study to evaluate the aggregation power of crystals in the absence (control) and presence of the inhibitor (flavonoid and phenol rich fraction of the plant). The results demonstrated that the fraction from the aerial parts composed of flavonoids and phenols was effective in inhibition of crystal aggregation (percentage inhibition: 67.14±1.84%).

The same group made an effort to evaluate the ability of *A. lanata* extract to hamper the growth of the existing calcium oxalate crystals (Mandal et al., 2017;2019). Interestingly the fraction rich in flavonoid and phenol effectively reduced the further growth of crystals. The observed findings explore the possible anti urolithiatic potential of *A. lanata* and explains the scientific rationale for prescribing the plant material to ameliorate the stones associated with the urinary system

3.1.2 In - vivo studies

Animal models were used to evaluate the in -vivo anti urolithiatic property in several studies. In all those studies, ethylene glycol in drinking water has been used to induce the renal calculi in animal models. The animals were treated with methanolic fraction of *A. lanata* flowers and noted that the serum and urine levels of stone forming substances such as calcium, phosphate, uric acid, oxalic acid and protein levels were significantly lower in test animals when compared to controls (Behera and Ghosh, 2018). The extract increased the urine volume, thereby reducing the solubility of calcium oxalate and other crystallizing salts such as uric acid, which may induce epitaxial deposition of calcium oxalate.

Potential anti urolithic activity of aqueous suspension of *A. lanata* was evaluated by Soundararajan et al., (2006). Oxalate synthesizing enzymes such as glycolic acid oxidase (liver) and lactate dehydrogenase (in liver and kidney) was significantly elevated in rats treated with ethylene

glycol. Administration of aqueous extract (2g/Kg body weight/ 28 days) to calcium oxalate urolithic rats demonstrated diminished levels of oxalate synthesizing enzymes. Further the test group showed reduced urinary excretion of stone forming substances such as calcium, oxalate, uric acid and phosphorus. On the contrary, increased concentration of inhibitors of stone formation such as citrate and magnesium were also evident in urine of urolithic rats treated with the extract. The study suggested that *A. lanata* could be used as a curative agent for urolithiasis.

Two isolated compounds (Quercetin and Betulin) of *A. lanata* were evaluated for anti-urolithiatic potential of calculi induced male Wistar albino rats by administering a test dose of 2 mg/kg BW orally for 28 days (Dinnimath et al., 2017). Urine microscopy revealed a significant reduction in the size of the calculi and significantly enhanced excretion of stone forming substances such as calcium, oxalate and phosphates in test animals. A significant reduction in the levels of blood urea and nitrogen was observed in rats of the test group exploring, that the kidney function of treated rats has presumed back to normal.

3.2 Diuretic effect

In CAM therapies, *A. lanata* is considered as a herbal plant with excellent diuretic properties (Jayaweera, 1981). In Sri Lanka, the plant's effectiveness as a diuretic has been studied by three research groups (Udupihille and Jiffry, 1986; Goonaratna et al., 1993, Herath et al., 2005).

When the plant extract was given to humans in concentrations of 50g /200 ml and 100g /200 ml, a significant increase in the urine volume was observed as compared with controls (Udupihille and Jiffry, 1986). In this study the diuretic property was determined by measuring the urine output.

Goonaratna et al., (1993) carried out a more descriptive study and measured the excretion of electrolytes (Na^+ and K^+) since most diuretics increase the electrolyte excretion together with urine output. However, contradictory results to the previous report were obtained by this study. The findings revealed that *A. lanata* does not produce diuresis, natriuresis, kaliuresis or change in urine osmoalar output although the reports did not mention the type of plant (dried or fresh) that was used to prepare the decoction.

Subsequently, Herath et al., (2005) conducted a preliminary study to investigate the diuretic effect of the fresh and dried parts of *A. lanata* by using rats. The concentrations of 50g/200 ml and 100g/200 ml of *A. lanata* were selected as therapeutic doses and the observed findings were in line with the first Sri Lankan study reported above (Udupihille and Jiffry, 1986). The findings showed that there was an increase in the urine output, urine osmolality and K^+ excretion in test rats during the four-hour observation period (Herath et al., 2005).

Another study from India reported that the isolated compounds Quercetin and Betulin from *A. lanata* can increase the urine output in urolithic rats and thereby shows the diuretic effect (Dinnimath et al., 2017).

3.3 Toxicological potential of *A. lanata* on kidney

Effect of isolated compounds Quercetin and Betulin on kidneys of urolithiatic rats were evaluated by Dinnimath et al., (2017). Electron microscopic examination of the kidney tissue of control (urolithiatic group) and test (urolithiatic rats treated with Quercetin and Betulin) rats were compared to ascertain the toxicological effects if any were produced by isolated compounds from *A. lanata.* Nevertheless, the treated group had shown intact histopathological features (glomeruli, proximal convoluted tubules and cellular organelles) when compared with deteriorated tissue architecture in calculi induced rats.

The first detailed experimental study to investigate the effect of *A. lanata* on the structure and function of the urinary tract was carried out by

a group of researchers from Sri Lanka using Sprague -Dawley rats (Gunatilake et al., 2012a; Gunatilake et al., 2012b). Two doses of dried *A. lanata* (25 g/200 ml and 100 g/ 200 ml) was used in the study. The authors justified that 25g/200 ml is the amount commonly prescribed by Ayurvedic physicians to prepare the infusion and that was selected as the low dose. The other dose was used as the high dose (four times the normal dose). The extract (dried whole plant) was administered orally for a period of one month. Light microscopic studies and electron microscopic studies were carried out to evaluate the effect of infusion on the structure of the kidney. Light microscopically visible changes were not seen in the glomeruli of kidney specimens when stained using hematoxylin and eosin, periodic acid schiff and silver methamine stains. However significant ultra-structural changes were observed in the epithelium of the proximal convoluted tubules when examined under the electron microscope. Disrupted brush border and altered mitochondria was observed in test rats and the authors claimed that this might affect the reabsorption of solutes from the tubular lumen leading to reduction in the passive absorption of water in the proximal convoluted tubule. Proximal convoluted tubule is responsible for reabsorption of 60-65% of the filtered water from the tubular lumen (Kumar and Clark, 2016). The authors suggested that increase in the urinary flow rate is correlated with damage to the epithelium of the proximal convoluted tubule. However, this study could be considered as an important study since the presence of ultra-structural changes is an early marker of the damage to the tubules. Electron microscopic changes are expected to occur prior to light microscopically detected histological changes.

In line with the evidences of the above study it is uncertain that diuresis produced by the extract of *A. lanata* is due to the damage which would occur in proximal convoluted tubule. In such a case the obligatory water resorption at the proximal convoluted tubule is impaired and therefore a diuretic effect is possible (Kumar and Clark, 2016).

The toxicological studies were carried out on rodent species and therefore the findings cannot be directly applied to humans. However, a human study of similar nature is also not feasible. Nevertheless, the findings of this important study cannot be neglected since, if a single study provides evidences of toxicity that has to be taken into consideration for the sake of the mankind.

Further, these information support the belief of the Ayurvedic physicians of not prescribing it for more than a week in the credence that it can damage the renal structure leading to renal failure in humans.

Another study conducted in Nigeria also reported the possible toxicological effects of prolong infusion of the plant extract to animal models (Kayode et al., 2017). In this study, the extract of *A. lanata* was orally administered to rats (daily at a dose of 40-1000 mg/kg for 90 days) to evaluate the sub chronic toxicity of the infusion. A significant increase in the weight of some organs such as lungs, brain and pancreas were reported in female rats. Haematological parameters were also altered (increased total leukocytes and neutrophils, reduced platelets) significantly in test rats. The extract has shown to be spermatotoxic (reduced sperm count and motility) in infused male rats exhibiting reduction in male reproductive capacity. The findings suggest that caution must therefore, be applied in its use on a long-term basis.

Conclusion

A. lanata has shown to inhibit stone formation and induce diuresis. Therefore, infusion of this plant for urinary incontinence and other disorders associated with urinary tract is very common in South Asian countries where Ayurvedic and traditional treatments prevail. Nevertheless, over nephro-protection, evidences are mounting towards its possible toxicity on kidneys and other tissues in animal models. Moreover, Chronic Kidney Diseases of Unknown Origin (CKDU) has become a significant health issue in many countries including Sri Lanka and greatly hamper the quality of life in elderly population. There

may be a greater possibility of use of *A. lanata* among these patients, though this fact has not been investigated scientifically. Therefore, those people who tend to self-medicate with *A. lanata* should be cautious in view of these findings, in a country like Sri Lanka where many people still depend on Ayurvedic and traditional systems of health care.

Conflicts of Interest: The author declares that there is no conflict of interest.

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Original Article

Anticandidal activity of ten selected medicinal plants from Southern and North Central provinces of Sri Lanka.

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Abstract

Candida is a commensal yeast which normally resides on the human body causing mild to serious infections and is the most frequent cause of fungal infections worldwide. Antifungals prescribed for the treatment of candidiasis have side effects and have become incompetent due to emerging resistance of fungi. Therefore, effective novel antifungals are required to overcome this obstacle. This study aimed to determine the anticandidal activity of selected medicinal plants used in ayurveda, against *Candida albicans* ATCC12420 and *Candida glabrata* ATCC 90030. Plants for the study were selected from Southern and North-Central provinces of Sri Lanka. Methanol extractions prepared from each plant were subjected to antifungal susceptibility testing (AFST) against both *Candida* species, according to the NCCLS guidelines. Fluconazole and dichloromethane were used as positive and negative controls respectively. The minimum fungicidal concentration (MFC) was determined for each plant extract by broth microdilution method. The initial concentration of 200 mg/mL of plant extract showed no clear zone of inhibition thus fungicidal activity could not be determined by disc diffusion method, however there was a reduced density of the lawn of growth with *Citrus aurantiifolia*, *Cinnamomum verum*, *Phyllanthus emblica* and *Psidium guajava* against *C. glabrata* and for *C. verum* against C. *albicans*. MFC was determined using doubling dilution of plant extracts with concentrations ranging from (500 mg/mL – 15.62mg/mL). A MFC of 31.25mg/mL for *C. albicans* were given by the plant extracts *C. verum, C. longa* and *P. guajava.* While a MFC of 31.25mg/mL was observed for *C. glabrata* with the plants extracts of *S. grandiflora*, *C. verum*, *P. emblica* and *P. guajava*. Leaf extracts of both *C. verum and P. guajava* have good antifungal activity against *C. albicans* and *C. glabrata*

Key words **-** *Candida* species, Antifungal susceptibility testing, Medicinal plants, Antifungal drugs, Minimum fungicidal concentration.

Introduction

Candida species are commensal flora found in skin, gastrointestinal tract and mucous membranes. (Toya et al., 2007) *Candida* species can cause mild to serious fungal infections (candidiasis) in human beings. It is the most common cause of fungal infections worldwide (Manolakaki et al., 2010). Incidence of candidiasis has significantly increased in the past three decades mainly due to increasing elderly population, rise of AIDS epidemics and immunocompromised patients (Rüping et al., 2008). Main cause for the candidiasis is *Candida albicans*, however non-*C. albicans* species such as *Candida glabrata*, *Candida tropicalis* and *Candida parapsilosis* are now frequently identified as human pathogens (Silva et al., 2012). Reported study findings suggest that about 75% of women develop vulvovaginal candidiasis at least once in their lifetime (Denning et al., 2018). Invasive candidiasis occurs when *Candida* species enter the bloodstream and spread throughout the body when immunity is declined. Germ tube or pseudohyphae formation is the major virulence factor of *Candida albicans* (Rathod et al., 2016).

Candida glabrata is a species in genus *Candida* which was initially emphasized as a nonpathogenic commensal of human mucosal tissues (Silva et al., 2012). However, with the extended use of immunosuppressive agents, mucosal and systemic infections caused by *C. glabrata* have increased significantly (Toya et al., 2007).

Although plentiful antimicrobial agents have been discovered, microorganisms are constantly developing resistance to these agents (Sharanappa & Vidyasagar, 2013). Studies revealed that *Candida albicans* has developed resistance to azoles and polyenes like Amphotericin-B (Irshad et al., 2011). Further antifungals are expensive and have side effects including toxicity (Sharanappa & Vidyasagar, 2013). Therefore, it is necessary to search for more effective and less toxic novel antifungal agents that would overcome these disadvantages (Fan et al., 2008). Plants generally produce many secondary metabolites which have properties like microbiocidal, pesticidal and have been increasingly used in pharmaceutical industry (Rathod et al., 2016). The positive and negative effects of plant extracts on fungi have been studied vastly by researchers from different parts of the world (Hire & Dhale, 2012).

Psidium guajava is a small tree that has been used traditionally as a medicinal plant and leaf extracts of *P. guajava* have been reported to have analgesic, anti-inflammatory, anti-microbial, hepatoprotective and antioxidant activities (Ryu et al., 2012).

Senna alata is an ornamental bush and it has been identified that the phytochemical components such as alkaloids, flavonoids, saponins, tannins, terpenes, anthraquinones, steroids and carbohydrates present in *Senna alata* contain antifungal properties as reported from a study in Nigeria (Owoyale et al., 2006).

Curcuma longa commonly known as turmeric, is traditionally used as a spice in Indian and Sri Lankan cuisine (Luthra, et al., 2001). A study done by Upendra et al, demonstrated that the turmeric has appreciable inhibitory action against fungal contaminations at the concentration of 0.8 and 1.0 g/L (Upendra et al., 2011).

Various parts of *T. indica* tree such as seeds, root, leaves, bark and fruits are used in traditional medicine in India and Africa as antifungal agents (Gunasena & Hughes, 2000).

Cinnamon extract is active against *Candida albicans*, and *Helicobacter pylori* infections. The antimicrobial property of the cinnamon given by eugenol and a derivative of cinnamaldehyde (Devikatte, et al., 2005).

Phylanthus emblica commonly referred as "AmLa" fruit has been traditionally associated with numerous health benefits including anti-microbial properties (Hire & Dhale, 2012).

In Sri Lanka, studies conducted on natural anticandidal agents are comparatively low. Hence, this study evaluates the natural remedies which are currently used in Ayurveda and traditional medicine in Sri Lanka, for healing superficial fungal infections. Moreover, this study screened the anticandidal action of ten selected medicinal plants against *Candida albicans* ATCC 12420 and *Candida glabrata* ATCC90030.

Materials and methods

Identification of plants

Herbarium specimens selected and listed in Table 1 were sent to the National Herbarium of Royal Botanical Garden in Peradeniya for ethnobotanical identification.

Extraction of plants

The selected infection-free healthy plant materials were cleaned thoroughly with distilled water twice and dried in a shade until they achieved a constant mass of 250g. The dried plant materials were ground to obtain powder form and soaked in absolute methanol for two days. After two days, plant extracts were filtered through filter paper. The filtrate was then evaporated to dryness using a rotary evaporator (Temperature - 40ºC and pressure - 540mmHg). Dried extracts were stored in a freezer for further testing (Sánchez et al., 2016).

Determination of the anticandidal activity using disc diffusion method

Anticandidal activity of the plant extracts were determined according to the methodology proposed by Sánchez et al. (2016) with minor modifications.

Briefly a mass of 0.2g of dried extract was completely dissolved in 1mL of dichloromethane (DCM). Filter paper disks with 6 mm diameter were obtained (Whatman No: 1) and soaked in 10µl of each plant extract separately (2mg/disk). *Candida* suspensions of $1 - 2 \times 10^8$ CFU/mL were prepared in sterile normal saline for each isolate and compared with 0.5 McFarland turbidity standards. Sabouraud dextrose agar plates were inoculated separately with *C. albicans* and *C. glabrata*.

Each plant extract-soaked disc was separately placed 3 cm apart on the culture plates. All ten plants-soaked discs were placed in a 150mm SDA culture plate inoculated with 0.5 McFarland turbidity *Candida* suspensions according to NCCLS method and incubated 48 hours at 35±2°C (Pfaller et al., 2002).

Zones of inhibition were measured in millimeter. All tests were duplicated.

As the positive control, fluconazole (25µg) antifungal drug was used. As the negative control, DCM soaked 6mm diameter (Whatman No: 1) filter paper disc was used.

Scientific name	Common name	Part of the plant used in the study
Psidium guajava	Guava	Leaf
Senna alata	Aththora	Leaf
Curcuma longa	Turmeric	Rhizome
Ricinus communis	Castor	Leaf
Cymbopogon citratus	Lemongrass	Whole plant
Tamarindus indica	Tamarind	Leaf
Cinnamomum verum	Cinnamon	Leaf
Sesbania grandiflora	Kathurumurunga	Leaf
Phyllanthus emblica	Indian gooseberry	Fruit
Citrus aurantiifolia	Lime	Leaf

Table 1: Ethno botanical data of plant species

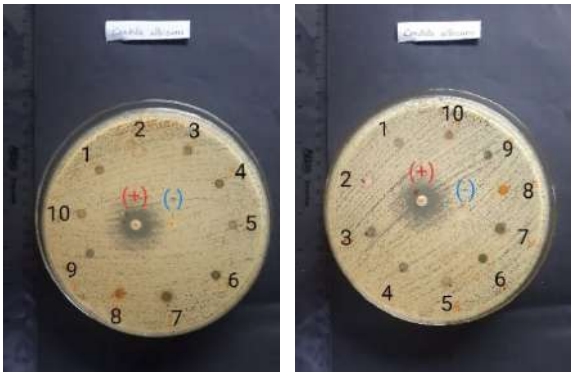

Figure 1: AFST for Candida albicans

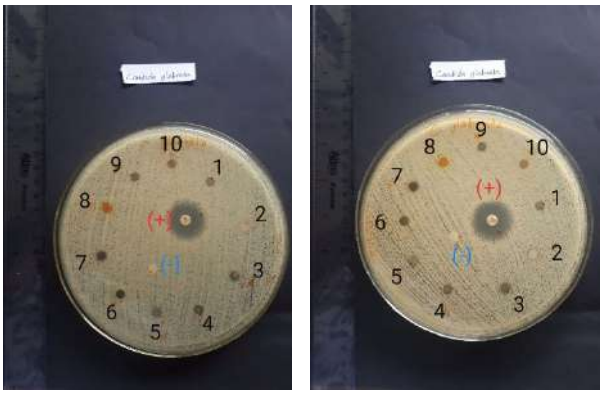

Figure 2: AFST for *Candida glabrata*

Sample number	Name of the plant extract
1	*Ricinus communis*
2	*Cymbopogon citratus*
3	*Tamarindus indica*
4	*Senna alata*
5	*Citrus aurantiifolia*
6	*Sesbania grandiflora*
7	*Cinnamomum verum*
8	*Curcuma longa*
9	*Phyllanthus emblica*
10	*Psidium guajava*
(-) control	DCM soaked disc
(+) control	Fluconazole (25µg)

Determination of minimum inhibitory concentration and minimum fungicidal concentration

All plant materials were further tested for minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Dilution series of plant extracts were prepared using a microtiter plate ranging from 500mg/mL to 15.63mg/mL. As the positive control and negative control 2mg/mL of fluconazole drug and 100 µl of DCM were used respectively. *Candida* suspension of 1 - 2 × 10^8 CFU/mL (0.5 McFarland turbidity) was prepared using sterile normal saline for each isolate. Loopful of *Candida* suspension was inoculated into each well and incubated for 48 hours at 35±2°C to detect minimum inhibitory concentration. After 48 hours MIC was to be detected visually, but due to the colour of plant extracts visual detection was not possible.

MFC was determined by subculturing loopful of plant extract-*Candida* suspensions on SDA agar, from the dilution tubes (500mg/mL – 15.62 mg/mL). Sub cultured plates were incubated at 35±2°C for 48 hours to detect viability.

All the experiments were performed in duplicate. Results were expressed as means along with the standard deviation (SD) of two parallel measurements.

Results

Anticandidal activity of plant extracts

There was no clear zone of inhibition around the discs tested compared to the control (Figure 2). Zone of inhibition is affected by several factors like thickness of the agar media, incubation time, pH, environmental factors and diffusivity of product (Flanagan & Steck, 2017). Hence another method to determine antifungal activity was used. MFC was determined for the following plant extracts.

1. *Citrus aurantiifolia*
2. *Phyllanthus emblica*
3. *Senna alata*
4. *Ricinus communis*
5. *Cymbopogon citratus*
6. *Tamarindus indica*
7. *Cinnamomum verum*
8. *Curcuma longa*
9. *Psidium guajava*
10. *Sesbania grandiflora*

MFC of all plant extracts tested against the *C. albicans* and *C. glabrata* are shown in the figure 3.

Interestingly both *C. verum* and *P. guajava* showed a MFC of 31.25mg/mL for both *Candida* species tested.

MFC of 125 mg/mL was seen for both *R. communis* and *C. citratus* which was the highest inhibitory concentration for both *Candida* species tested. *C. verum* and *P. guajava* showed low fungicidal activity when compared to the above two plant extracts and gave a MFC of 31.25mg/mL.

Further, 125mg/mL MFC was observed with *S. grandiflora, P. emblica,* and *T. indica* against *Candida albicans*. Interestingly the MFC of 31.25 mg/mL was seen with both *S. grandiflora and P. emblica* against *C. glabrata* while 62.5 mg/mL was obtained for *T. indica* against *C. glabrata.*

A MFC of 62.5 mg/mL for *C. glabrata* was seen with *C. aurantifolia and C. longa*, while a MFC of 62.5mg/mL and 32.25mg/mL was seen with *C. aurantifolia* and *C. longa* respectively against *C. albicans.*

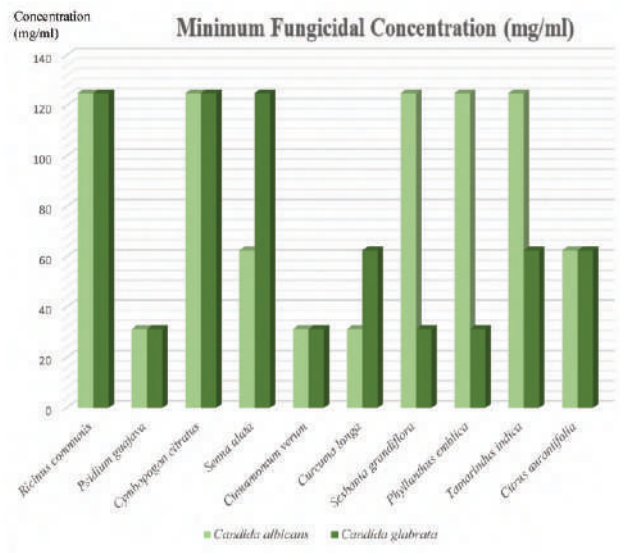

Figure 3: MFC of *Ricinus communis, Psidium guajava, Cymbopogon citratus, Senna alata, Cinnamomum verum, Curcuma longa, Sesbania grandiflora, Phyllanthus emblica, Tamarindus indica and Citrus aurantifolia*

Extract of *S. alata* showed a MFC of 62.5mg/mL and 125mg/mL respectively for *C. albicans and C. glabrata*.

However, *R. communis, C. citratus, T. indica, S. alata, S. grandiflora* and *C. aurantifolia* did not reveal any antifungal activity to the plants tested.

Discussion

Increasing resistance to antifungal agents is indeed a global problem. Further the toxicity and side effects to antifungal is yet another concern. Therefore, the use of natural products as alternative agents for the control of fungal disease is considered as an interesting alternative to synthetic fungicides.

As seen in this study both plant extracts taken from leaves of *P. guajava* and *C. verum* had good fungicidal activity (31.25 mg/mL) against both *Candida* species. Similarly studies from India also reported that the two concentrations (50mg/mL and 25mg/mL) of bark of the *C. verum* aqueous extracts had markedly inhibited several *Candida* species including *C. albicans* and *C. glabrata* (Vinitha & Ballal, 2008).

In keeping with the study findings, another study done in Northern Brazil from leaves of *P. guajava* had also shown to have a significant inhibitory activity against non- *Candida albicans* species when compared to *C. albicans* (Ferreira et al., 2013).

C. longa is another medicinal plant with known antibacterial and anticandidal activities. Current study revealed that the rhizome of the *C. longa* had potential anticandidal activity at low concentrations (31.25mg/mL) when tested against *C. albicans*. A previous study revealed that MIC of the DCM extracts of *C. longa* completely inhibited *C. albicans* at a concentration of 512 µg/mL (ÇıKrıkçı et al., 2008) showing that turmeric *(C. longa)* can be active at very low concentrations.

In the disc diffusion method, the inhibitory zones of respective plant extracts were not clear, and it could be due to poor diffusion of the plant extracts across the agar plate. Promising results have been seen when performing the MFC testing for *C. verum a*nd *P. guajava* against both *Candida* species tested.

In this study, part of the plant was only experimented for anticandidal effect.

Chemical composition of the plant differs according to the part of the plant, season of the year, climate, geographical variations and the age of the plant and thus could have varying activity. The selection of the parts of the plants for native treatment vary from geographical region to region. However as seen in this study the leaves of *C. verum, P. guajava, S. grandiflora, T. indica, S. alata* and rhizome of *C. longa* and fruit of *P. emblica* have seen to be effective and comparable with other reported studies (Gul & Bakht, 2013), (Vinitha & Ballal, 2008), (Kumar et al., 2021), (Zohrameena et al., 2017).

Further phytochemicals are antimicrobial in nature, but they also produce other biological activities in vivo resulting in induction of immunity, which can indirectly reduce the risk of infections (Packiyalakshmi et al., 2016).

According to the phytochemical analysis, previous studies revealed that *Cinnamomum verum is* rich with many phytochemical ingredients, such as cinnamic acid, cinnamaldehyde, cinnamate, and numerous polyphenols (Batiha et al., 2020).

Considering the phytochemical properties, leaves of *P. guajava* is rich with flavonoids such as quercetin, avicularin, apigenin, guaijaverin, kaempferol, hyperin, myricetin, gallic acid, catechin, epicatechin, chlorogenic acid, epigallocatechin gallate, and caffeic acid (Kumar et al., 2021). Hence these plants could prove to be useful alternatives to western medicines as they possess both antifungal and other properties which could curtail infection.

Conclusion and recommendations

Leaves of Sri Lankan cinnamon *(Cinnamomum verum)* and guava (*Psidium guajava)* have good antifungal activity against both *Candida* species tested. Thus, both plant species can be used as alternatives to traditional topical preparations for superficial *Candida* infections. Further investigations are needed to identify the active compounds from the active fractions of the extract.

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Conflicts of interests

There are no conflicts of interest.

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Original Article

Awareness on Efficacy and Side Effects of Female Contraceptives among Nursing Undergraduates at KIU, Sri Lanka.

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Abstract

Unplanned pregnancies have rapidly emerged as a social issue that significantly degrades the quality of life of parents and children. Therefore, nurses, being a vital part of the health system, should be well-aware regarding contraceptive methods. The objective of the study was to assess awareness of safety, efficacy, and side effects of female contraceptives among nursing undergraduates at KAATSU International University (KIU). Descriptive cross-sectional study was conducted among 362 undergraduate nurses studying at KIU, Sri Lanka, using a convenient sampling method. The data were collected using a self-administered pre-tested questionnaire that consisted of four parts; demographic variables, safety, efficacy and side effects related questions. The data was analyzed using descriptive statistics and the Pearson Chi-square test. Out of total, 74.6% (n=270) of the participants had an average awareness regarding different types of female contraceptives and 74.5% (n=270) had average awareness (40-59.9%) regarding major side effects of female contraceptives while the awareness regarding efficacy of different contraceptive methods was found to be only 37% (n=134) related to female condom. Overall, the study revealed that majority of nurses had an average awareness regarding the safety and main side effects of female contraceptive methods, further they had a low level of awareness on efficacy of female contraceptive methods. Therefore, it is important to enhance nurses' awareness related to modern female contraceptive methods.

Keyword: Undergraduate nurses, Female contraceptives, Awareness, Efficacy, Side effects

Introduction

Contraceptives have been practiced for thousands of years globally. Many women used it for an extremely long period throughout their reproductive lifespan (Monga & Dobbs, 2011). People use different types of contraception at different stages in their lives and there is no single method that will suit everyone. There is no perfect method of contraception and different types of contraceptive methods will have several advantages and disadvantages (Monga & Dobbs, 2011). Ideal contraceptives will be characterized by low cost, high efficacy, and minimal side effects.

The ability of a woman to start a successful, continuous and appropriate contraceptive method is influenced by many factors; e.g. access to the health care, community, cultural attitudes, and personal attitude all of which can be considered as obstacles to applying correct use and efficient technique to achieve family planning goals (Blumenthal et al, 2010; Belfield, 2009 & Mohammed, 2019). Furthermore, in developing countries, where women are dependent upon old traditions and social constraints, awareness, and awareness about family planning acceptance is not the only decisive factor.

However, lack of information or misinformation about various techniques, can confuse and discourage people from taking any form of contraception. Also, some women are prevented from using any contraception by a partner or are unable to access services because of their youth or unmarried status. Anyhow, in many cases, these obstacles can be overcome through proper contraceptive education. It is a known fact that education could improve understanding and make the person think scientifically (Joshua et al, 2014).

In Sri Lanka, a large budget is being allocated for the public health system to ensure that the majority of the population has access to primary health care (Family Health Bureau, 2016). According to 2016 statistics, the total population of Sri Lanka was 21,164,458, and the birth rate per 1000 of the population was 16.90, while mortality rate was 5.80 per 1000 live births. The major reason identified for neonatal mortality is congenital anomaly which stands at 42.6% and majority of Sri Lankan mothers had good awareness regarding congenital anomalies (Kanchana & Youhasan, 2018). Through planned pregnancy, the rate of congenital defects would have been reduced. During the last few decades, family planning assisted many couples in planning their reproductive lives (WHO, 2014 & Ministry of Health, 2016). These results are seen in the form of a steep reduction in mortality rates, especially maternal and infant mortality, along with a decline in fertility rates. This reduction in population leads to the sustainable development of the country.

According to an article published by Perera et al, in 2004, inadequate, inaccessible, and unaffordable Family Planning (FP) services and social barriers that prevent women and couples from using FP methods may be responsible for a substantial proportion of unwanted pregnancies. Even when FP services are available and accessible, a proportion of unwanted pregnancies arise following contraceptive failure due to incorrect use. Sri Lanka appears to have a well-established family planning program (Family Planning, 2010). The primary objective of the programme was to reduce unwanted births by improving family planning services across the island, obviating the necessity for illegal abortions. As a result, health care professionals play a critical role in the delivery of these services (Demographic & Health Survey Report, 2016).

The tendency to use contraceptive methods depends upon the individual's general attitude toward using those methods. Hence knowing individuals' attitudes will be important. As long as people's attitudes are known, their conduct may be expected and managed. Further, anticipating

and managing behaviors are extremely important for nurses. Contraceptive consulting is more than providing information or answering the questions of the clients, therefore, nurses must explore and modify their attitudes and beliefs in this area (Blumenthal et al, 2010). Choosing a contraceptive method for any couple is an extremely important part of reproductive health.

Nurses can assist women in achieving their reproductive life goals, such as spacing and timing of children, by providing efficient contraception counseling. However, lack of awareness, misconceptions, and negative attitude towards contraceptives in nursing personnel can act as a barrier for their personal use and also prevent them from promoting contraceptives to the beneficiaries. However, relatively a few published studies on contraceptive methods and services have been conducted in Sri Lanka. Therefore, the objective of the of the study was to assess the awareness on safety, efficacy, and side effects of female contraceptives among nursing undergraduates at KAATSU International University (KIU).

Methodology

A descriptive cross-sectional study design was used among 362 registered, undergraduate nursing students studying in the 3rd and 4th year at KIU, Sri Lanka. The sample size was calculated using Cochran formula (estimate prevalence-50%). The data were collected using a self-administered pretested questionnaire which consisted of four parts. The questionnaire was developed by the researcher using the available literature. The first part included eight questions related to demographical data, the second part was designed to assess the awareness of female contraceptive methods which consisted of six questions, the third part was designed to assess the awareness on efficacy rate, and it consisted of two main questions with several subcategories and the fourth part was aimed to assess the awareness regarding side effects of female contraceptives (Oral Contraceptive Pills-OCP, Intrauterine Device - IUD), depo-provera, implant and female condom) which included thirteen questions. Data was collected through an established database at KIU using convenient sampling techniques. After obtaining the ethical clearance from Ethics Review Committee, KIU (KIU/ERC/18/010), a pilot study was conducted among 26 nursing undergraduates apart from the main study to identify the validity and reliability (Cronbach's alpha = 0.75) of the questionnaire. After the pilot study, the questionnaire was modified accordingly. Data were analyzed in SPSS version 23 and descriptive statistical analysis was performed to describe the data. Pearson Chi-square test was used to determine the association between the categorical variables. The awareness of participants was assessed by an awareness assessing scale developed by the researcher. According to this scale, the participants were graded as; if scored less than 39.9% were categorized as a poor level of awareness, between 40-59.9% as an average level of awareness, and 60% and above having as a good level of awareness.

Results

Demographic characteristics of the participants

Among the 362 participants, the frequency percentage of demographic variable shows majority (40.6%, n=147) were from the Western province and 58.8% (n=213) were between the age of 26-30 years, while the majority (96.1%, n=345) were Buddhist. According to the Sri Lankan nurses grading circular, the majority (87.3%, n=316) of the nurses were ranked as nursing officer grade III. Majority (20.7%, n=75) worked at a medical ward and most of them (47.2%, n=171) had 1-5 years of working experience. Most of the participants (89.5%, n=324) did not have any special training regarding family planning .

Awareness regarding female contraceptive methods

The awareness related to various female contraceptive methods were assessed including Oral Contraceptive Pills (OCP), IUD, Depo-Provera, Implant, and Female condom. Majority (92.5%, n=335) were aware that OCP pack contain 28 pills with 21 hormonal pills. When questioned on "Pills can be taken every day but at any time" and "OCP can prevent total fertilization process," the responses gained were 64.6% (n=234) and 68.8% (n=249) respectively indicating inadequate awareness. Further it also indicated that the majority were unaware of OCP's effect on the fertility process and that it should be taken every day at the same time. In response to the statement "IUDs are reversible and can get pregnant after taking the device out" 93.7% (n=339) responded correctly and 34.8% (n=126) answered that the statement of "After IUD insertion, intercourse must be avoided for 1 week" as true. This statement concluded that the majority of the participants were unaware that they can have intercourse after 24 hours.

According to the awareness regarding Depo-Provera it was found that the majority did not have appropriate awareness regarding the statements of that the "Depo-Provera reduces the risk of endometrial cancer" and "Depo-Provera cannot be used longer than two (02) years" since majority 56.1% (n=203) and 56.4% (n=204) had responded to these statements incorrectly. This highlights that participants were unaware that Depo-Provera can reduce the risk of endometrial cancer and it cannot be used longer than two (02) years. Furthermore, it was found that the majority (97%, n=351) responded correctly to the statement "It is inserted under the skin of upper arm". Only 36.5% (n=132) responded correctly to the statement "Implants can be inserted immediately after miscarriage". Further, the minority 44.8% (n=162) and 46.1% (167) were aware that antibiotics like rifampicin can make the implant less effective and that an implant is effective for almost five (05) years. Most participants have appropriately answered the statements regarding female condoms. It was found that overall all the participants managed to obtain above 50% regarding all the statements. This highlights that the majority (94.8%, n=343) were aware that female condoms provide protection against pregnancy and STDs.

Awareness regarding efficacy of female contraceptive methods

On assessing the efficacy of female contraceptive methods, it was found to be relatively poor. The majority were unable to answer the statements given related to the efficacy. Out of 362 participants who responded correctly to the statements included 8.6% (n=31) for OCP (efficacy rate of 99%), 1.1% (n=40) for IUD (more than 99% effective) & Depo - Provera (efficacy rate of 99.6%), 5% (n=18) for implant (effective more than 99% with perfect use) and 37% (n=134) for female condoms (When used correctly all of the time, 95% effective). This highlights that the majority were unaware of the efficacy of different female contraceptive methods.

Table 01, depicts the computed chi-square value for the level of awareness regarding different types of female contraceptive methods. It was found to be statistically significant as the p-value is less than the set p-value (0.05) among all the methods. In conclusion, there was a significant relationship between awareness and all the female contraceptive methods. The participants were graded as; if scored less than 39.9% were categorized as a poor level of awareness, between 40-59.9% as an average level of awareness, and 60% and above having as a good level of awareness.

Table 01: Association between overall awareness and types of female contraceptive methods

Statements	Good Awareness level	Average awareness level	Poor awareness level	P value
awareness on OCP	23.2%	74.6%	2.2%	0.001
awareness on IUD	83.4%	11.6%	5%	0.001
awareness on Depo-provera	35.9%	28.7%	35.4%	0.001
awareness on implant	35.9%	36.2%	27.9%	0.001
awareness on female condom	48.1%	37.3%	14.6%	0.001

[$p \leq 0.05$ and Confidence Interval (CI) = 95%]

Awareness regarding side effects of female contraceptive methods

On assessment of side effects of female contraceptive methods majority stated that breast tenderness is a side effect of OCP (61.3%, n=222) and Depo-provera (50.8%, n=184) while 58.3% (n=211) stated vaginal infection is a side effect of IUD, 19.1% (n=69) stated weight gain as a side effect of implants and 23.8% (n=86) stated allergy as a side effect of female condoms. However, 74.5% (n=270) of the nurses had average level of awareness regarding side effects on female contraceptive methods. Furthermore, 74.5 % (n=270) had average awareness of the side effects of female contraceptive methods, while 2.2 % (n=8) and 23.3 % (n=84) had good and poor awareness, respectively regarding side effects of female contraceptive methods. Further the analysis showed that there were significant association between overall awareness and side effects (p=0.001) of different female contraceptive methods ($p \leq 0.05$).

Discussion

Since nursing personals are an integral part of any health care system, they act as a reliable source of information for the general public. Nurses frequently provide basic contraception care and guidance in primary care settings and further, refer women for specialized assistance (Kelsey, 2017). In addition to that, awareness regarding awareness, and positive attitudes toward family planning activities among eligible women are strongly advocated, and healthcare workers, particularly nurses and doctors should have sound awareness, and positive attitudes towards family planning (O'Driscoll & Parrott, 2019).

In the current study the majority of the participants (74.6%) had average awareness regarding the female contraceptive methods and only 2.2% had a good level of awareness. However, 23.2% were considered as having poor awareness. It is possible that the higher percentage of nurses with average awareness is attributable to the fact that not all nurses are trained to be midwives or that they have not worked in a healthcare setting that specializes with reproductive health. As a result, they will have less opportunity to learn extensive information about "female contraceptives." Awareness plays an important role in motivating contraceptive methods towards family planning (Bamufleh et al, 2017 & Charandabi et al, 2012). The current study showed low level of awareness in OCP (23.2%), injectable progesterone (35.9%), and implant (35.9%) except IUD (83.4%) when compared to the study conducted by Shahid in 2018 among nursing and midwifery students. According to that study although participants had a good understanding of the various methods, such as oral contraceptives (62%), injectable progesterone (63%), implants (83%), intrauterine contraceptive device (37%), tubal ligation (48%), and vasectomy (78%), their attitudes toward reliable contraceptives were not as positive as natural methods (Shahid, 2018). A study conducted regarding the awareness of emergency contraception among future healthcare providers in Northern Ghana found that almost more than half (54.9%) of the participants were unaware of emergency contraceptives (Mohammed, 2019). Another study conducted related to the awareness and attitude on emergency contraception among nursing personnel shows that an average, nearly three fourth (72.83%) of nursing personnel had awareness on emergency contraception as a whole

(Thapa, 2013). Furthermore, the awareness, attitudes, and practice of health care providers influenced the provision of contraceptive care to adolescents in Botswana (Tshitenge et al, 2018). Based on that, the majority of health care persons were only providing contraceptives on an irregular basis and were unfamiliar with newer contraceptive approaches (Tshitenge et al, 2018).

Moreover, according to the current study, nurses' awareness regarding the efficacy of female contraceptive methods concluded that the majority of the participants were not able to answer the statements regarding the efficacy of female contraceptive methods. The current analysis of data illustrated, only 37% were aware of the efficacy rates of female condoms, 8.6% regarding OCP, and 5% were aware of implants. It was also observed that only 1.1% were aware of the efficacy of IUD and Depo-Provera. However, a study done by Charandabi et al (2012) on communicating contraceptive effectiveness found comparatively higher level of awareness on efficacy of female contraceptive methods compared to our study. As the results Charandabi et al (2012) found that, only 46% of women knew that Combined Oral Contraceptives (COC) are more effective than condoms and 50% knew that IUDs are more effective than condoms. Furthermore, in a study on communicating contraceptive effectiveness, 83% of women said they consulted with a health care provider for the effectiveness of contraceptive methods (Steiner, 2003). In addition to that, Agasti in 2017, showed despite of having adequate awareness among female health workers, the usage of contraceptives by eligible couples in their respective locations was low due to a lack of motivational skills among them and some religious misconceptions and social norms. Therefore, this highlights the importance for nurses to have proper awareness regarding the efficacy of contraceptives methods.

The findings of the current study revealed that the majority of nurses had average awareness (74.5%) regarding the major side effects of female contraceptive methods. It was also found that 2.2% of nurses had a good level of awareness on side effects of female contraceptive methods. However, 23.3 % were considered to have inadequate awareness. A survey of Vietnamese medical students revealed that they have a high level of awareness, perceptions, awareness, and practice regarding contraceptive methods (Nguyen & Vo, 2018). Furthermore, a study conducted by Perera et al, in 2004, regarding awareness, behavior, and attitudes on induced abortion and family planning among Sri Lankan women illustrated 45% had been introduced to family planning by the public health midwife and among them out of 159 participants, a significant number, 30 had discontinued the family planning method due to socio-cultural reasons and adverse effects. Another study conducted about contraceptive prevalence in Qatar shows that 15.9% of women who currently used contraceptives, reported experiencing one or more side effects. The most common side effects in the Qatar study are vaginal bleeding bleeding (4.1%), severe headache (3.1%), abdominal pain (2.3%), and vaginal discharge (2%) (Arabab et al, 2011). On contrary, the current current study on awareness among nurses reported breast tenderness, vaginal infection, and weight gain as the major side effects. In addition to that, it has been shown, that the rate of discontinuation is higher among women who have not been adequately counseled about side effects (Arabab et al, 2011 & Sato et al, 2020). Therefore, nurses can play a vital role in counseling the community about the side effects of different female contraceptives.

In the current study there was no significant association found in awareness level regarding the awareness on contraceptive safety with the age, and years of experience as a nurse. In addition to that, a significant relationship was found between awareness and special training

regarding family planning (p=0.019). Arabab et al (2011) found that women who were currently on contraceptives had a significant association with their age, partner's age, years of being married, educational status, economic status, and attitudes on family planning, which was similar to our findings.

Conclusion

This study concludes that majority of the nurses had average awareness regarding safety and major side effects of female contraceptive methods. However, nurses have poor awareness regarding the efficacy of female contraceptive methods. Furthermore, the level of knowledge regarding how to take OCP properly and its action on fertilization process was low. The awareness level was significantly associated with age and years of nursing experience. It can thus be concluded that it is critical to provide nurses with proper information and training in female contraceptive techniques during their career.

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Original Article

Medicinal plants used in cancer treatment: A survey conducted among traditional Ayurveda medical practitioners in Sri Lanka

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Abstract

Majority of deaths worldwide have been caused by noncommunicable diseases, with cancer as the second leading cause. Plant-based cures have become increasingly popular because current synthetic pharmaceuticals and other medications have demonstrated severe side effects, leading to less patient compliance and treatment failure. Furthermore, for most cancer types, there is no permanent cure. Sri Lankan traditional practitioners employ natural plant remedies to treat and cure malignancies, drawing on a long history of Ayurveda treatments and ancestral wisdom. These practitioners use different extraction processes, different parts of the same plant, and different modes of administration of the same herb. This descriptive cross-sectional study identifies commonly utilized botanicals with anticancer properties by traditional ayurvedic practitioners in Sri Lanka. The snowball approach and purposive sampling were used to select medical practitioners for this study. The survey was conducted among 18 traditional ayurvedic practitioners, and from the survey, 120 plants that the practitioners used for cancer therapy were identified. Of those 120 plants, the repetitive plants were shortlisted for ten plants. The most commonly used plants identified in this survey were *Flueggea leucopyrus, Curcuma longa, Aegle marmelos, Abrus precatorius, Phyllanthus emblica, Zingiber officinale, Annona muricata, Aloe vera, Manihot esculenta*, and *Solanum melongena*. Most medicinal plants used for cancer treatment were from the family Fabaceae.

Keyword: Anticancer, Medicinal plants, Sri Lanka, Traditional medical practitioners

Introduction

Cancer is a significant health concern that contemporary medicine must cope with. In 2020, there were 19.3 million cancer cases and 10 million cancer deaths (World Health Organization, 2020a). According to the World Health Organization, cancer claims the lives of 16,691 people in Sri Lanka yearly (World Health Organization, 2020b). The annual cancer death rate in 2020 was higher than in previous years (World Health Organization, 2018). Chemotherapy, radiation, and surgery are the only three major cancer treatments available in modern medicine (Jesmin & Sarker, 2014). Modern medicine has improved efficacy and increased survival but also has unpleasant side effects. Furthermore, they do not appear to be 100% effective. As part of a new strategy, the scientific community focuses on traditional medical practice to develop a viable solution (Nurgali, Jagoe, and Abalo, 2018).

Sri Lanka has had a rich indigenous knowledge and cultural repertory related to traditional medicine for over 3000 years (de Zoysa & Palitharathne, 2008). Ayurveda, Sidda, Unani, and Desiya Chikitsa are today's four essential components of traditional medicine. The Desiya Chikitsa is the indigenous component and also apparently the oldest (Arseculeratne, 2002). Sri Lanka is a tropical country with abundant natural herbs and a great history of traditional plant-based medicine, and alternative therapy identification Traditional ayurvedic medicine will reduce the country's healthcare burden (Jones & Liyanage, 2018). Increased attempts have been made in the last several decades to isolate bioactive chemicals extracted from herbs, which have been known for their utility in synthesizing novel medications that are less toxic and more effective against non-tumor cells than synthetic drugs (Shafi et al., 2018).

Medicinal plants have been providing essential therapeutic assistance in treating people's ailments for thousands of years. More than 60% of anticancer medications are derived from natural ingredients (Khor et al., 2018). As a result, herbal medicines have received attention worldwide in recent years. Plant extracts have displayed many critical biological activities (Mishra, Kumar, and Pandey, 2013). Different phenolic compounds found in herbal plants and are known to contain anti-cytotoxic properties have the potential to fight against cancer rather than synthetic drugs (Turrini et al., 2018).

The importance of analyzing the scientific basis of herbal medicine for treating various ailments is growing daily, with the enhanced attention to traditional and folk medicine (Metel, 2017). Traditional medicine has its methods for obtaining beneficial components from these plants. Because they are natural, the chances of adverse effects are minimal (Sofowora et al., 2013). However, there is a significant gap in understanding between the plants employed in traditional anticancer treatments and their mode of action methods. Further, introducing traditional Sri Lankan medicine to the scientific society is challenging due to the unwillingness of the traditional Ayurveda practitioners to share their knowledge (Silva, 2016). The traditional knowledge is buried with the practitioner most of the time. Therefore, this study aims to broach the hidden traditional knowledge of Ayurvedic practitioners and present it to the modern scientific world. Further, this study aims to emphasize current scientific evidence by reviewing selected traditional medicinal plants.

Methodology

Study Area and Selection of Respondents

This survey was conducted from May to December 2019 among traditional Ayurvedic practitioners in Sri Lanka who engaged only in cancer treatment. Twenty - one registered traditional Ayurvedic practitioners were identified in cancer, cancer & balaroga, and cancer boils subcategories as per the Ayurvedic council categorization (Council, 2021). Snowball and purposive sampling techniques were used to conduct a descriptive cross-sectional study on

traditional Ayurvedic medical practitioners who practiced cancer treatments. Each practitioner was informed of the study's objectives before data collection to obtain their consent and cooperation for the survey. However, the sample size was limited to eighteen practitioners as some contacted practitioners were unwilling to share their traditional Ayurvedic knowledge for the study.

Preparation of Questionnaire

This survey was conducted by using an interviewer-administered questionnaire. Informed consent was obtained for the survey from eighteen medical practitioners. Information related to herbal plants for anticancer therapy was collected from the practitioners via a pretested questionnaire. Data was collected via a semi-structured questionnaire. The first section was designed to gather general information about the practitioners, including the practitioner's name, gender, age, experience in the profession, education level, and province/district. Other sections of the questionnaire were mainly dedicated to gather information on medicinal plants and plant parts used in cancer treatments. The part of the plant used for the medicines, effective dosages, method of administration, method of obtaining authentication of plants, extraction methods of plants were also collected. However, information on cancer remedies and particular cancer types were not collected due to the unwillingness of practitioners to disclose their family recipes. The data regarding the active components of the plant and its association with different cancers were derived through a literature survey (Table 03).

Ethical approval was obtained from the Ethics Review Committee of KIU (KIU/ERC/19/31). The collected data were tabulated and analyzed using Statistical Package for the Social Sciences software 16 to evaluate descriptive statistics of the population**.**

Results

From the selected sample of 18 traditional ayurvedic practitioners, the majority were from Anuradhapura 4(22.2%), Colombo 4(22.2%), and Kandy 4(22.2%) Districts.

According to the study data, 17 medical practitioners were registered Ayurveda doctors with degrees and diplomas in science. One practitioner was not registered under the Ayurvedic council but practiced traditional Ayurveda.

Among the 18 medical practitioners, 11(61.1%) medical practitioners were female. Other than cancer treatment, these practitioners practiced as general practitioners (sarwanga), treated patients for, orthopedic disorders (kadum bidum), ayurvedic surgery, diabetes, and for gynaecology disorders. These practitioners used their experience (education, clinical trials, ancestral manuscripts, Ayurveda literature books, Olam books) to authenticate the plants used for their ayurvedic treatment. None of the ayurvedic practitioners used novel techniques such as botanical authentication by a botanist.

When inquired into the treatment method used, 11(61.1%) of the practitioners were using external body treatments (skin application, panchakarma prathikarma, paththu badeema and herbal bath), and 7(38.9%) of the practitioners used internal body treatment via traditional preparations (kasaya, chuurna, guli, kalka, arishta and drugs) as cancer treatments.

When the patient assessment was inquired into, 13(72.2%) practitioners confirmed their diagnosis by observing patients' signs and symptoms, 3(16.7%) of the practitioners came to their diagnosis by discussing with the patient, 1(5.6%) used pulse assessment, and only 1(5.6%) used laboratory reports for their diagnosis.

Analysis of responses revealed details about 120 plants that are used in cancer treatment during recent times by the traditional Ayurvedic

practitioners in the survey. Among them, the majority (18 (15%)) of plants were from the family Fabaceae, followed by Apocyancea (07(5.83%)) and Rutaceae (06 (5%)) as indicated in figure 1. According to the survey results, the plants of the Fabaceae, Apocyancea and Rutaceae families are the most commonly used plants for cancer treatment in this report. The names of the plants used for cancer belonging to the most commonly used families are indicated in table 1.

Table 1 – Plants used for the cancer treatment belonging to the most commonly used plant families

Family	Plants
Fabacea	*Erythrina variegate, Bauhinia tomentosa, Clitoria ternatea, Desmodium triflorum, Trigonella foenum, Vigna radiate, Cicer arietinum, Macrotyloma uniflorum, Bauhinia purpurea, Abrus precatorius, Cassia auriculata, Mucuna pruriens, Mimosa pudica, Canavalia cathartica thouars, Canavalia ensiformis (Linn), Caesalpinia sappan, Glycyrrhiza glabra*, and *Cassia fistula*
Apocynaceae	*Hemidesmus indicus, Catharanthus roseus, Rauvolfia serpentine, Rauvolfia densifolia, Calotropis procer, Dregea volubilis*, and *Nerium oleander*
Rutacea	*Aegle marmelos, Citrus reticulate, Acronychia pedunculata, Limonia acidissima, Citrus aurantium*, and *Feronia limonia*

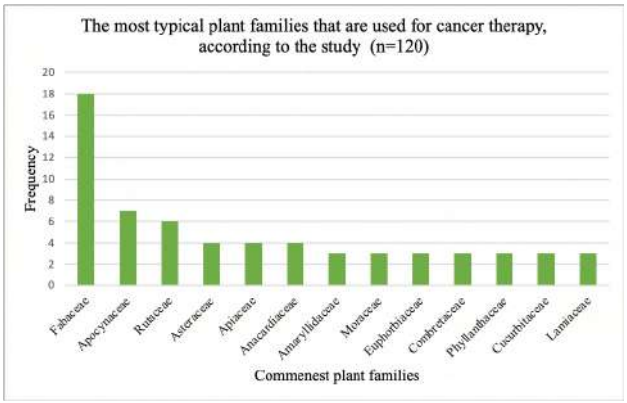

Figure 1 - The most typical plant families that are used for cancer therapy

Further, the most commonly used 10 plants among the 120 plants were selected for further review. Accordingly data gathered from the 18 medical practitioners revealed that *Flueggea leucopyrus* (n=13), *Annona muricata* (n=7), *Curcuma longa* (n=6), *Aegle marmelos* (n=4), *Abrus precatorius* (n=5), *Phyllanthus emblica* (n=5), *Zingiber officinale* (n=4), *Aloe vera* (n=4), *Manihot esculenta* (n=4), and *Solanum melongena* (n=4) were the most commonly used plants in the study (Figure 2).

A - *Aloe vera*, B - *Zingiber officinale*, C - *Curcuma longa*, D - *Annona muricata*, E - *Abrus precatorius*, F - *Phyllanthus emblica*, G - *Solanum melongena*, H - *Manihot esculenta*, I - *Flueggea leucopyrus*, J - *Aegle marmelos*

Figure 2 – The ten most commonly used plants by the 18 medical practitioners of the ten most commonly used plants, *Fluggea leucopyrus*, *Annona muricata*, *Curcuma longa* were seen to be used more commonly than others.

The part of the plant of the ten most commonly used plants is shown in table 2. According to the received data majority of the medical practitioners are using *F. leucopyrus* as the whole plant for cancer treatment (8(61.5%)), while the leaves and roots were used by 3 (23%) and 2 (15.3%) practitioners respectively. *A. muricata* was used by seven medical practitioners and the majority (5 (71.4%)) used the fruit for cancer treatment while 1(14.2%) practitioner used the leaves and 1(14.2%) practitioner used the whole plant for the treatment. When inquired about *C. longa*, all (6(100%)) the practitioners used the rhizome. *A. marmelos* was used by five medicinal practitioners, on inquiry into the parts of the plants used, fruit, roots and immature leaves were used by 2(50%), 1(25%) and 1(25%) respectively. *A. precatorius* leaves were used by 3(60%) and seeds were used by 2(30%) practitioners for the treatment of cancer. When considering *P. embellica* 2 (40%) used the dry fruit, 2 (40%) used the leaves and 1(20%) used the whole plant for treatment. *Z. officinale* rhizome was used by 3(75%) and leaves were used by 1(25%) of the medical practitioners in anticancer therapy. *Aloe vera* leaf gel was used by 3(75%) and the whole leaf was used

by 1(25%) in the treatment. *Manihot esculenta* tuber was used by 3(75%) and leaves were used by 1(25%).

Table 2 – Part of the plants used in the treatment of cancer for the most commonly used plants.

Vernacular name in Sinhalese	Scientific name	Family	Part of the plant used in the treatment by the traditional
Katupila	*Flueggea leucopyrus*	Phyllanthaceae	Leave (n=3) Whole plant (n=8) Roots (n=2)
Katu anoda	*Annona muricata*	Annonaceae	Fruit (n=5) Leaves (n=1) Whole plant (n=1)
Kaha	*Curcuma longa*	Acoraceae	Rhizome (n=6)
Beli	*Aegle marmelos*	Rutaceae	Immature leaves (n=1) Roots (n=1) Fruit (n=2)
Olinda/ Gunja	*Abrus precatorius*	Fabaceae	Leaves (n=3) Seeds (n=2)
Nelli	*Phyllanthus emblica*	Phyllanthaceae	Dry Fruit (n=2) Leaves (n=2) whole plant (n=1)
Iguru	*Zingiber officinale*	Zingiberaceae	Rhizome (n=3) Leaves (n=1)
Komarika	*Aloe vera*	Xanthorrhoeaceae/ Aloaceae	Leaves (n=1) Leaf gel (n=3)
Manyokka	*Manihot esculenta*	Euphorbiaceae	Tubers (n=3) Leaves (n=1)
Ela Batu	*Solanum melongena*	Solanaceae	Roots (n=1) Fruit (n=3)

Following literature survey on the association between the ten most commonly used plants, their active compounds and type of cancer treatment table 3 was generated. It was revealed that Katupila both fresh and dry were used to treat liver cancer, endometrial cancer, breast cancer, prostate cancer, ovarian cancer and acute leukaemia, while *C. longa* was used in the treatment for a larger number of cancer types (table 3). Interestingly breast cancer was treated with all ten plants listed in table 3. Further, literature revealed that lung cancer was treated with *Curcuma longa*, *Aegle marmelos*, *Abrus precatorius*, *Phyllanthus emblica*, *Annona muricata* and *Aloe vera.* Only two plants *Zingiber officinale* and *Annona muricata* were found to be effective against pancreatic cancer.

Table 3 – The active compounds and the targeted cancer type for the most commonly used ten plants

No	Vernacular name in Sinhalese	Scientific name	Family	The physical nature of the plant used	Active compound/ promising anticancer agent	Scientific claim for the authentic use: targeted cancer types	References
01	Katupila	*Flueggea leucopyrus*	Phyllanthaceae	Fresh Dry	Bergenin, gallic acid quercetin, kaempferol, coumarin	• Liver cancer • Endometrial carcinoma • Breast cancer • Prostate cancer • Ovarian cancer • Acute Leukaemia	(Soysa et al., 2014) (Wijayabandara et al., 2015) (Samarakoon et al., 2014) (Deepika et al., 2018) (Muhammed et al., 2018)
02	Kaha	*Curcuma longa*	Acoraceae	Dry	Alpha (α)-and beta (β)-asarone	• Oral cancer • Gastric cancer • Glioblastoma • Colorectal cancer • Gastric cancer • Prostate cancer • Cervical cancer • Lung cancer • Breast cancer • Neuroblastoma • Lymphoma • Liver cancers • Lymphoma	(Das et al., 2019) (Antony et al., 2017) (Haghighi et al., 2017) (Sreejaya & Santhy, 2015) (Sharma et al., 2020)
03	Beli	*Aegle marmelos*	Rutaceae	Fresh Dry	Furanocoumarin imperatorin, Marmelin, lupeol, eugenol, citral, cineole and d-limonene	• Breast cancer • Liver cancer • Lung cancer • Colon cancer • Ovarian cancer • Prostate cancer • Leukemia	(Seemaisamy et al., 2019) (Akhouri et al., 2020) (Vardhini et al., 2018) (Bhatti et al., 2013) (Murthy et al., 2020) (Kumar & Bodla, 2018)
04	Olinda/ Gunja	*Abrus precatorius*	Fabaceae	Fresh Dry	Stigmasterol hemihydrate, (β-monolinolein	• Cervical cancer • Breast cancer • Cervix cancer • Leukemia • Ovarian cancer • Prostate cancer • Colon cancer • Liver cancer • Lung cancer • Oral cancer	(Wan-Ibrahim et al., 2019) (Okoro et al., 2019) (Sofi et al., 2018) (Patil et al., 2015) (Panda, 2013)
05	Nelli	*Phyllanthus emblica*	Phyllanthaceae	Fresh Dry	Ellagic acid, Corilagin, Pyrogallol, Chebulagic acid, Gallic acid, Quercetin	• Cervical cancer • Lung cancer • Colon cancer • Liver cancer • Ovarian cancer • Colorectal cancers • Breast cancer	(Kuruppu et al., 2019) (Zhao et al., 2015) (Mahata et al., 2013) (Asmilia et al., 2020) (VERMA et al., 2012) (Ngamkitidechakul et al., 2010) (Zhao et al., 2016)
06	Iguru	*Zingiber officinale*	Zingiberaceae	Dry Fresh	Gingerols, which are converted to shogaols, paradols and zingerone	• Colorectal cancer • Breast cancer • Liver cancer • Cervical cancers • Pancreatic cancer • Gastrointestinal cancer	(Kuruppu et al., 2019) (Ansari et al., 2016) (Park et al., 2014) (Akimoto et al., 2015) (Prakash et al., 2016) (S. Prasad & Tyagi, 2015)
07	Katu anoda	*Annona muricata*	Annonaceae	Fresh Dry	Acetogenins	• Breast cancer • Colorectal cancer • Skin cancer • Head and neck cancers • Lung cancer • Liver cancer • Pancreatic cancer • Prostate cancer • Bone cancer • Ovarian cancer • Cervical cancer • Leukemia	(S. K. Prasad et al., 2019) (Cassé, 2018) (Agu et al., 2018)
08	Komarika	*Aloe vera*	Xanthorrhoeaceae/ Aloaceae	Fresh Dry	Aloe-emodin	• Skin cancer • Liver cancer • Leukemia • Breast cancer • Gastric cancer • Lung cancer • Oral cancer • Prostate cancer • Bladder cancer • Cervical cancer • Colon cancer • Nasopharyngeal cancer • Oral cancer • Ovarian cancer	(Sanders et al., 2018) (Candöken et al., 2017) (Shalabi et al., 2015) (Yonehara et al., 2015)
09	Manyokka	*Manihot esculenta*	Euphorbiaceae	Fresh	Linamarin, β-carotene, vitamin C, and fiber	• Leukemia • Breast cancer • Colon cancer	(Diana et al., 2018) (Mustarichie et al., 2020) (Nisa et al., 2015) (Arafa et al., 2016)
10	Ela Batu	*Solanum melongena*	Solanaceae	Fresh Dry	Glycoalkaloids (solasonine, solasodine and solamargine), steroidal glycosides (β-sitosterol-3-O-β-D-glucoside and poriferasterol-3-O-β-D-glucoside)	• Liver cancer • Colon cancer • Larynx cancer • Breast cancer • Cervix cancer • Liver cancer • Skin cancer	(Fekry et al., 2019) (Sarah & Misbahuddin, 2018) (Shabana et al., 2013)

Discussion

As revealed by the current study majority of the medical practitioners treating cancer, cancer & balaroga, and cancer boils were females. In keeping with those study findings, the Basement Report of the Institution Frame of Private Sector of Western Medicine and State Indigenous Medicine Sector 2017 reports that most consultants/specialists are females, working full-time and part-time in Sri Lanka. Further compared to males the distribution of female consultants in the state indigenous medicine sector is 74% (Ministry of Health and Indigenous Medicine, 2017). Comparable to this study other studies in Mali (Nordeng et al., 2013), Indonesia (Peltzer & Pengpid, 2019) and Canada (Ijaz et al., 2021) have shown a higher number of traditional female practitioners.

Rather than relying on cutting-edge technology, Sri Lankans Ayurveda and traditional medical practitioners continue to draw on their expertise to manage cancer patients by consulting Olam books, ancestral manuscripts, and Ayurveda literature books as found in this study. Similarly, analysis into global ayurvedic practices has seen a staling progression due to predominant

heritage, pride and past glory base perception among traditional ayurvedic practitioners and the reluctance to practice evidence based medicine (Patwardhan, 2014).

According to the study data, 17 medical practitioners were registered Ayurveda doctors with degrees and diplomas in science. One practitioner was not registered under the Ayurvedic council but practiced traditional Ayurveda for over two decades passing down generations. It is evident that many ayurvedic practitioners practice without registrations not only in Sri Lanka but globally (Welch, 2008).

Identifying the correct medicinal plants that go into preparing a medicine is essential in the ayurvedic medicinal industry (Jayanka & Fernando, 2020). Alarmingly it was noticed that the study population selected in this report practiced using plants based on traditional experience and did not resort to additional authentication or scientific method in identifying plants.

According to this report, most plants used by traditional ayurvedic practitioners for cancer treatment are from the family Fabaceae. Herbs from the Fabaceae family have traditionally been used to treat various ailments. Isoflavones, lectins, saponins, and phenolic compounds are among the phytochemicals found in the Fabaceae family. Sebastian and Gomathi report that the phytochemicals present in Fabaceae family act as antioxidants and are used for treatment and prevention of cancer (Sebastian & Gomathi, 2020). Further the family Fabaceae is well known for its anticancer property and has been extensively reviewed by the scientific community (Oliveira et al., 2022). According to Sharma et al., those who consume plants of the family Fabaceae as a staple diet have lower cancer mortality (Sharma et al., 2017).

As reported in the current study, *Flueggea leucopyrus* has been used extensively in treatment of cancer in Sri Lanka (Hettihewa et al., 2015). Current study determined that the practitioners used whole plant, roots and leaves. Bulugahapitiya et al., 2020 has reported that the leaves of the *F. leucopyrus* plant contains a Bergenin isomer which is responsible for the anticancer activity (Bulugahapitiya et al., 2020). Anticancer activity also has been detected for the bark and aerial parts of this plant(Bulugahapitiya et al., 2020). Further report suggest that bergenin could be used to develop more potent galectin-3 inhibitors which is a anticancer mechanism (Jayakody et al., 2018, Stegmayr et al., 2019). As determine by the literature survey done in this report the aerial parts of the *F. leucopyrus* is effective against the breast cancers (Mendis et al., 2015) and endometrial carcinoma (Samarakoon et al., 2014). Further the bark of *F. leucopyrus* has been identified to be effective against ovarian carcinoma (Hettihewa et al., 2015)

Annona muricata was the second most commonest plant (fruits, leaves and whole plant) used for cancer therapy in this study group. Other studies have shown that leaves of *Annona muricata* have potent anticancer activity (Naik et al., 2021) and also has been used in breast cancers (Gavamukulya et al., 2014). The most important phychemicals of *A. muricata* are alkaloids, phenols and acetogenins (Coria-Téllez et al., 2018). According to Rady et al, in Africa and South America several parts of the plant including the bark, fruits, leaves, pericarp, seeds, and roots, have been used to treat a variety of cancers breast, prostate, colorectal, lung, leukemia, renal, pancreatic, hepatic, oral, melanoma, cervical, and ovarian cancers (Rady et al., 2018).

Studies have found that *C. longa* is a commonly used anticancer plant as determined in this study. The major ingredient in *C. longa* is curcumin. It is reported to inhibit various cancers such as colon, hepatocellular, breast, renal, prostate cancers, T cell leukemia, and B cell lymphoma (Aggarwal & Chandra Bharti, 2003).

Several studies have highlighted the importance of plant extracts as anticancer agents in Breast cancer (Levitsky & Dembitsky, 2015; Mitra &

Dash, 2018). Interestingly the current study identified that all th ten commonest plants have been used as breast cancer therapy. This highlights the importance of plant extracts as a future product to fight the battle against cancer. It further highlights the importance of proper authentication of the plant and in practicing evidence-based medicine.

Conclusion

The study concludes that *Flueggea leucopyrus, Curcuma longa, Aegle marmelos, Abrus precatorius, Phyllanthus emblica, Zingiber officinale, Annona muricata, Aloe vera, Manihot esculenta*, and *Solanum melongena* are commonly used in cancer treatment.

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