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QUALITATIVE ASSESSMENT OF PHYTOCHEMICALS OF ETHANOLIC – AQUA EXTRACT OF *PITTOSPORUM VIRIDIFLORUM* LEAVES

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ABSTRACT

Pittosporum viridiflorum is used to treat stomach complaints, chest pain, malaria and other fever. As per our previous studies of antibacterial activity of ethanolic extract of the *P. viridiflorum*, it was active against *E. aerogenes, E.coli, Proteus vulgaris, Bacillus cereus* and *S. typhi*. The main objective of the research work was to analyse the phytochemical constituents of the ethanolic - aqua extract of *P. viridiflorum* leaves. From this analysis the extract was found to contain saponins, phytosterols, phenols, flavonoids and diterpenes. The important phytochemicals present in the plant leaves help to fight against above revealed microorganisms. Hence, this plant is a scientific justification use in the traditional treatment against various diseases affecting human beings.

KEYWORDS: Phytochemical, pharmacological, leaves, ethanol, Pittosporum and viridiflorum.

INTRODUCTION

Previous studies on the antibacterial activity of ethanolic extract of the *P. viridiflorum have shown that* it was active against *E. aerogenes, E.coli, Proteus vulgaris, Bacillus cereus* and *S. typhi* (Anthoney, 2014). The main aim of our present study was to analyse the phytochemical constituents of the ethanolic extract of *P. viridiflorum* leaves.

The charming *Pittosporum viridiflorum* (Cheesewood) is a useful evergreen tree with an attractive dense, straight or rounded crown and lovely glossy deep green foliage. The Cheesewood varies in size and shape depending on where it is planted and can be maintained as a small tree of about 4 m or left to grow to its full height. These delightful trees are irresistible to insectivorous birds when the sweetly fragrant flowers appear, along with a host of insects, while a wide variety of seed eating birds such as the red eyed dove, flock to the tree when the startlingly bright red seeds appear. *P. viridiflorum* is a truly excellent all rounder and is becoming increasingly popular as a garden and street tree throughout the country (Treeco, 2012).

Phytochemicals are naturally occurring components in the medicinal plants that have defense mechanism and protect from various diseases (Krishnaiah, 2007). The medicinal plants found to have several pharmacological uses such as antibacterial activity, antifungal activity, anti-diabetic activity, anticancer activity, antioxidant activity, hepatoprotective activity, haemolytic activity, anti-inflammatory activity, larvicidal activity, anthelmintic activity, central nervous system activity and pain relief activity (Mir, 2013 and Sukirtha, 2012). Allopathic medicines have many side effects and in order to counteract these effects, many people are now turning to nature in pure form to prevent and cure diseases using natural medicinal herbs (Deshpande, 2010).

Research on medicinal plants is of great importance and taking into account the old and new problems emerging day by day. Medicinal plants are available in nature and our ancestors have information about their medicinal value traditionally (Prajapati, 2003). With increase in diseases caused by the modes of leaving and emerging drug resistant microbes, back -to-nature is becoming a common acronym to many people in the world today. The use of plants in the past tells clearly the fascinating relationship between mankind and plants since ancient times. Due to lack of clear knowledge on the mode of treatment of certain plants, people in the past have attributed the healing of diseases using medicinal herbs to supernatural forces due to their indisputable healing capability (Dobelis, 1986).

According to Ameyaw (2009), many plants in Africa such as *Cassia siame (*Lam), *Nauclea latifolia (*Benth), *Cryptolepis sanguinolenta* (Lindl), *Azadiracha indica* and *Jatropha gossypifolia* have been tested for antimalarial properties. Many of the traditionally used plants in the African continent tested have shown great potential in biological activity (Gbeassor, 1963).

Medicinal plants have since ancient times been used to treat many illnesses which affect humankind even today. Many traditionalists have done this for quite some time and therefore prevented many deaths in the past few decades. However, this has been done with little scientific proof on the efficiency and the effects of the extracts on the affected individuals. Herbal medicine is still a matter of argument in the current world with many still doughting its efficiency. This has been due to greedy practitioners who want to become wealthy by pretending to know much about the diseases which their clients claim to have, hence leading to the application of wrong treatment and administration of wrong drugs which do not cure the patient and therefore leading to worsening of the situation or even death of the victim (Kokwaro, 2009).

The medicinal plants possess multiple medicinal properties, hence, enabling them to have several uses in the pharmaceutical industry. Studies on several plants have been done all over the world and plants have shown great potential in the treatment of diseases affecting both humans and animals. Study report on potentilla fulgens have shown the plant to have anti-hyperglycemic, hypoglycemic, anti-hyperlipidemic, antitumor, antioxidant, anti-inflammatory and anti-ulcerogenic properties (Koul, 2007).

Pittosporum is used as a source of wood for firewood, for charcoal, for engraving and, due to the large amount of nectar; it is recorded as good for honeybees. As a medicinal plant, only one record was found for Portugal: on Madeira, the whole plant crushed and applied in poultices is said to repair muscles, tendons and ligaments strained or torn by violent movement (Rivera, 1995 and Nicolau *et al*., 2007)

The Cheesewood has a number of medicinal properties and the bark as well as the roots has traditionally been used for a variety of ailments. Infusions of the bark are used to treat stomach complaints and fever, easing pain and having a generally calming effect. The powdered root is believed to have an aphrodisiac effect and is sometimes added to beer. The wood is pale and soft but is sometimes used for kitchen utensils (Treeco, 2012).

Pittosporum viridiflorum used as an emetic. The bark is also boiled in water, the decoction added to soup and then stirred, and finally drunk as remedy for chest complaints, malaria and other fevers. The liquid is bitter and induces violent vomiting (Kokwaro, 2008).

The present study was carried out to analyses the phytochemical of ethanolic extract of *Pittosporum viridiflorum* leaves.

MATERIALS AND METHODS

Sample collection and Extraction procedure: The leaves of the *Pittosporum viridiflorum* were collected around Baraton University campus. The samples were identified by a taxonomist in the University of Eastern Africa, Baraton. The fresh leaves of the *Pittosporum viridiflorum* leaves were air – dried for three weeks; the dried leaves were ground into powder. Forty grams (40 g) of the powdered leaves were mixed with 400 ml of ethanol – water (70:30). The mixture was kept for 24 hours on a shaker for effective extraction of the plant components. The extract was filtered and the solvent was evaporated to dryness at a temperature of 40^{o}C using rotary vacuum evaporator. The extract was brought to dryness using vacuum and pressure pump. The yield was kept at 4^{o}C prior to use.

Qualitative phytoconstituents analysis: The extracts phytoconstituents analysis for identification of bioactive chemical constituents was done using standard procedures (Trease, 1989; Harbome, 1973 and Sofowara, 1993).

1. Tannins

About 0.5 g of the sample was put in a test tube and 20 ml of distilled water was added and heated to boiling. The mixture was then filtered and 1 % of $FeCl_3$ was added to the filtrate and observations made. Brownish green coloration indicates the presence of tannins.

2. Saponins

The extract was mixed with 5 ml of water and vigorously shaken. The formation of stable form indicates the presence of saponins.

3. Flavonoids

A portion of the aqueous extract was added in a test tube. To this, 5 ml of dilute ammonia and 2 ml of concentrated sulphuric acid were added. The appearance of a yellow color indicated the presence of flavonoids.

4. Terpenoids

The extracts of the plant material was taken in a clean test tube, 2 ml of chloroform was added and vigorously shaken, then evaporated to dryness. To this, 2 ml of concentrated

sulphuric acid was added and heated for about 2 minutes. A grayish color indicates the presence of terpenoids.

5. Glycosides

Salkowsks' test: The extract of the plant material was mixed with 2 ml of chloroform and 2 ml of concentrated sulphuric acid was carefully added and shaken gently, then the observations were made. A red brown colour indicates the presence of steroidal ring (glycone portion of glycoside).

6. Alkaloids

The crude extract was mixed with 1% of HCl in a test tube. The test tube was then heated gently and filtered. To the filtrate a few drops of Mayer's and Wagner's reagents were added to the test tube. A resulting precipitate confirms the presence of alkaloids.

7. Steroids

Liebermann Burchard reaction: About 2 g of the extract was put in a test tube and 10 ml of chloroform added and filtered, 2 ml of the filtrate was mixed with 2 ml of a mixture of acetic acid and concentrated sulphuric acid was added along the side of the test tube. Blue green ring indicate the presence of steroids.

8. Phenols

The plants extract was put in a test tube and treated with a few drops of 2% of $FeCl_3$, blue green or black coloration indicate the presence of phenols.

RESULTS AND DISCUSSION

Table 1: Phytochemical analysis of ethanolic-aqua extracts of *Pittosporum viridiflorum* Leaves

Phytochemicals	Observation	Inferences
Carbohydrates	-VE	-VE
Glycosides	-VE	-VE
Saponins	+++	+
Phytosterols	+++	+
Phenols	+++	+
Tannins	-VE	-
Flavonoids	+++	+
Amino acids	-VE	-
Diterpenes	+++	+
Alkaloids	-VE	-

The phytochemical tests of the ethanolic aqua extract of ***Pittosporum viridiflorum*** leaves revealed the presence of saponins, flavonoids, , phenols, diterpenes and phytosterols [Table 1]. The presence of saponins shows the potential of the plants to be used to produce mild detergents and intracellular histochemistry staining to allow antibody access to intercellular proteins. They have been found to treat hypercholesterolemia, hyperglycemia, antioxidant, anti-inflammatory, central nervous system activities, anticancer and weight loss (Trease, 1989). According to Maobe (2013), saponins are used to stop bleeding, treating wounds and ulcers as it helps red blood cells to precipitate and coagulate. This can be attributed to ability of saponins to bind with glucose and cholesterol molecules. Saponins have also being associated with inhibitory effect on inflammation (Maobe, 2013).

Saponins are used by the folkloric remedies of Kashmir (India) in treating wounds (Foster, 1990), this is because of their ability to cause red blood cells coagulation and therefore help in blood clotting, treating wounds and enteric ulcers problems (Chiej, 1984). Saponins have also been used to prevent hypercholesterolemia and antibiotic activity, anti-inflammatory and anti-diabetic.

Flavonoids are used as antioxidants because of their ability to scavage free radicals such as peroxide and hydroperoxide of lipid hydroxyl hence inhibiting oxidation that lead to degenerative diseases (Samatha, 2012). They can be used as anti-diabetic. According to Namki (1999), flavonoids can be used to prevent synthesis of off flavours that are caused by fat oxidation. Flavonoids have been found to have antibacterial activity due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Yadav, 2011). Flavonoids are produced by plant in response to microbial infection and studies have shown that they have antibacterial activity against a wide range of micro-organisms (Ghasemzadeh, 2011).

Flavonoids are secondary metabolites with polyphenolic structure and synthesized in plants, through polypropanoid pathway (Sharma, 2006). Flavonoids have been classified in to six sub-groups which include flavones, flavanol, flavanone, flava-3-ols, isoflavone and anthocynidin. Flavonoids are known to contain specific compounds called antioxidants which protect human, animal and plant cells against the damaging effects of free radicals. Imbalance between free radicals and antioxidants leads to oxidative stress which has being associated with inflammation, autoimmune diseases, cataract, cancer, Parkinson's disease, aging and arteriosclerosis. It also plays a role in heart diseases and neurodegenerative diseases.

Flavonoids have also vasodilator activity a property which is useful in improving blood circulation in brain and in Alzheimer disease (Argal, 2006). Leaf extract of *Ginkgo biloba* which contains flavonoids was used for improving blood circulation in brain varix. Several isoflavone can be used to improve blood circulation. Furanocoumarins can alter hexobarbital induced sleeping time, showed cytotoxic action and inhibited growth of tumor in mice. Free radicals including the hydroxyl, hydroperoxide, superoxide and lipid peroxide have being associated with a number of diseases such as cardiovascular disease, cataracts, diabetes, gastrointestinal inflammatory diseases, cancer, asthma, liver disease, macular degeneration, periodontal disease and other inflammatory processes. These oxidants are produced during normal body chemical processes. They can be damaged through free-radical damage. Flavonoids such as quercetin, rotin, catechin and its derivatives and the oligomeric proathocyanidins (OPCS) have shown in vitro studies to inhibit the oxidation of low-density lipoproteins (LDL).

Phenols are present in fruit and vegetables which have received considerable attention because of their potential antioxidant activity. Human consumption of antioxidants has many alleged health benefits, including protection against cardiovascular diseases and cancer (López-Vélez, 2003). According to Syed (2011), phenols are spread in nature and are probably the largest group of secondary plant metabolites. They often exist in glycosidic forms and range from simple structures having a simple aromatic ring to highly complex polymeric structures (Akinmoladun, 2007). Phenols can be divided into several classes. The antibacterial activities of plants are due to simple phenolic compounds. Simple phenolic compounds consist of a single phenolic ring and often possess alcohol, aldehyde and carboxylic acid groups. Capsaicin is found in the dried ripe fruit of different species of Capsicum and have been used internally for dyspepsia and flatulence and externally, it is frequently used as counterirritant (Lotito, 2006).

According to Kuba (1992), the study of antimicrobial activity of six diterpenoids isolated from the bark of *Podocarpus nagi* (Podocarpaceae) has been tested against twelve selected microorganisms. Totarol, the most abundant compound among the six, exhibited potent bactericidal activity only against Gram-positive bacteria, among which *Propionibacterium acnes* was the most sensitive bacterium. Totarol also showed strong activity against four other Gram-positive bacteria tested: *Streptococcus mutans, Bacillus subtilis, Brevibacterium ammoniagenes,* and *Staphylococcus aureus* (both penicillin-resistant and penicillin-

susceptible strains). The bactericidal activity of totarol was enhanced when it was tested in combination with several other natural products. Similar antibacterial activity was observed when diterpene totarol and diterpene derivatives such as ferruginol, 6,7 dehydroferruginol, and acetylferruginol against gram positive and gram negative bacteria. Those seven diterpenes were tested against *Aspergillus sp., Fusarium fujikuroi, F. ciliatum, Mucor miehei, Nematospora coryli, Penicillium notatum and Paecilomyces variotii*, six of them exhibit activities. From these diterpenes, totarol and ferruginol are the most active (Becerra, 2002). The wood resistance to fungi and bacteria of Chilean Podocarpaceae is due to the high concentration of phenolic diterpenes (Becerra, 2002) and these results also agree with the observations made by Cambie *(1984)* on the resistance to fungi of the Fijian Podocarpaceae woods.

The antimicrobial activities of the phytosterols against various microbes were tested and the effects of pH and temperature on the antimicrobial activity against *Staphylococcus aureus* were evaluated. The phytosterols has strong inhibitory effects against *S. aureus* and *Bacillus subtilis* with the MICs of 18 μg/ml and 30 μg/ml, respectively, and has weak inhibitory effects against *E.coli* and *B. cereus*, but has no inhibitory effect against *Aspergillus niger*. The antibacterial activity against *S. aureus* is strongest when pH value is 5 - 6, and would not be affected in a certain temperature range (CHOU et al., 2009).

The petroleum ether extract of the leaves of *Annona squamosa*, *Adenocalymna alliceum*, and *Amaranthus tricolor* yielded *n*-alkanes, *n*-alkanols, 16-hentriacontanone, and sterols. These were evaluated for their antibacterial activity against *Staphylococcus aureus*, *Staphylococcus albus*, and *Streptocorus viridans* (all gram-positive bacteria) and *E.coli*, *Pseudomonas pvocyanea*, and *Klebsiella* (all gram-negative bacteria). Among the compounds isolated from these plants, 16-hentriacontanone and sterols exhibited stronger antibacterial activity (Sharma, 1993).

In our previous studies (Anthoney, 2014), on the antibacterial activity of *Pittosporum viridiflorum* leaves extract against laboratory strains of selected microorganisms showed that the mean zones of inhibition of the extract against microorganisms were 12.67±0.882 mm for *Enterobacter aerogenes*, 12.50±0.281mm for *Escherichia coli*, 11.67±0.333 mm for *Proteus vulgaris*, 11.67±0.000 mm for *Bacillus cereus* and 7.67±0.333mm for *Salmonella typhi*. Analysis of variance showed that the zones of inhibition of the extract and antibiotic control against the microorganisms were significantly different. Tukey's honestly test further showed

both significant and non-significant comparisons between the extract and controls for various bacterial organisms. These potent antibacterial activities might be due to naturally occurring bioactive phytochemicals present in this plant. It could be concluded that detailed characterization of various compounds from leaves of *Pittosporum viridiflorum* is needed, so that structure activity relationship in term of antibacterial activity and antifungal activity could be investigated.

CONCLUSION

This study suggest that the ethanolic extract of *Pittosporum viridiflorum* leaves were found to contain saponins, phytosterols, phenols, flavonoids and diterpenes. The presence of these important phytochemicals in the plant leaves is a scientific justification of the use of the plant in the traditional treatment against various diseases affecting humans and animals. Apart from the chemical methods which employ the use of solvents to get the active compounds, scientist also need to prepare the extracts just the same way they are prepared traditionally in order to justify the indisputable capability of pants to treat against various diseases, scientist also needs to investigate the specific active compounds in the infused plant extracts and their medicinal value.

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