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Pharmacognostic and preliminary phytochemical investigation on leaf extracts of Pavonia zeylanica Cav.

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Abstract

The study is aimed at development of Physico-chemical parameters and to investigate the active principle present in *Pavonia zeylanica Cav*. (Malvaceae) commonly known as Ceylon Swamp Mallow and in Kannada it is called as Topala, Balarakshasi, and Antutogari. The Ceylon Swamp Mallow is a profusely branched, bristly, large herb, growing up to 1-1.5 m tall. The Plant extract is used for vomiting, vermifuge, oliguraia, tumours and fever**.** Therefore, from extensive literature survey it was revealed that no reports were available on chemoprofile of *Pavonia zeylanica* to check the identity and purity of the drug. The present work embodies the investigations carried out to establish methods for quality control of drugs as per WHO guidelines: physico-chemical parameters like Loss on Drying, Extractive values, Foaming index, Ash values and to investigate the phytochemical present in the extracts in the preliminary level were also carried out for the quality control of the drug. Thus it was thought worthwhile to explore this plant on the basis of its standardization parameters. The study will provide referential information for the correct identification of the crude-drug.

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INTRODUCTION

Plants have an almost limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as the molecules of plant defence against predation by microorganisms, insects, and herbivores. Further, some of which may involve in plant odour (terpenoids), pigmentation (tannins and quinines), and flavour (capsaicin).

However, several of these molecules possess medicinal properties[1].

Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial, and antiviral activities. The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing, and in recent years, there has been a worldwide trend towards the use of the natural phytochemical present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables[2].

Pavonia zeylanica is a plant that has shown potential as a source of chemotherapeutic compounds. Phytochemical studies have revealed that the plant is rich in flavonoids and other water soluble polyphenolic compounds. This present study, therefore investigated the physicochemical and phytochemical compositions of this plant.

MATERIALS AND METHODS

Plant Material

A survey was conducted during 2007 to 2008 in Nanjangud area of Karnataka to collect the plant material of *Pavonia zeylanica*. Botanical species were collected from the forests of Nanjangud area, Mysore district, Karnataka, voucher specimens were collected, identified properly consulting a flora and the same has been deposited in the Department of Botany, University of Mysore, Mysore-06.

Leaves were shade dried and powdered to 40-mesh size. The physicochemical parameters like extractive values, florescence characteristics of powdered leaf and leaf extract, preliminary phyto-profiling and phytochemical analysis, were determined as per WHO guidelines1. The average percentage w/w of the ash content and the extractive values were determined. The Fluorescence analysis was carried out according to the reported method[3,4] wherein the colour of the powdered leaf and leaf extract were also studied under ordinary and ultra-violet light at 366nm. Powdered leaf material was successively extracted with Petroleum ether, Benzene, Chloroform, Acetone, methanol, ethanol and water in soxhlet apparatus and was subjected for identification of various plant constituents[5,6].

Extraction of plant leaf material

The powdered plant leaf material was subjected to successive solvent extraction taking from polar to non-polar solvents like water, ethanol, methanol, acetone, chloroform, benzene and petroleum ether. 20gms of powdered plant material was subjected to soxhlet extraction for 8 hrs with 250ml of the various solvents. The extracts obtained were later kept for evaporation to remove the excessive solvents. These extracts were stored in a cool dry place for the analysis for the presence of preliminary phytochemicals.

Analysis of primary and secondary metabolites in the extracts of *Pavonia zeylanica*

The primary metabolites like; proteins, carbohydrates and fixed oils and fats, were analyzed for their presence as per the standard procedures[7, 8]. Similarly, the secondary metabolites like, alkaloids, flavonoids, saponins, phenolics, tannins volatile oils, terpenoids and glycosides were also assessed in the leaf extracts of *Pavonia zeylanica*. All the data generated from the study were subjected to arithmetic mean with standard deviation for statistical analysis.

RESULTS AND DISCUSSIONS

The extracts of *P.zeylanica* was also analyzed for Physicochemical values which were determined and the observed results were tabulated and statistically analyzed (Table No. 1,2,3,4,5 & 6). The determination of ash value for percentage of total ash, acid insoluble ash, sulphated ash and water soluble ash were 5.66, 0.64, 6.99 and 1.09 %w/w of leaves respectively. Extractive values of water soluble and alcohol soluble were observed to be 11.21 and 22.51 %w/w of leaves respectively (Table.1).

Table 1: Physicochemical characterization of leaf of *P. zeylanica*

Sl. no.	WHO Parameters	Average values %w/w of Leaves
1	Total ash	5.66
2	Acid insoluble ash	0.64
3	Water soluble ash	1.09
4	Sulphated ash	6.99
5	Alcohol extractive value	21.51
6	Water extractive value	11.21
7	Loss on drying	4.5

The parameters for the analysis of leaf of *P.zeylanica* were also carried out wherein the hemolytic activity for methanolic extract showed less than 100mg/ml of activity whereas water extract showed 8.05mg/ml of activity. The foaming index was found to be less than 100. The total tannin content was 1%. The total bacterial and fungal count was $7\times10^3$and 6×10^4 respectively. *E.coli* was present showing 5×10^3 whereas *Salmonella typhii* was absent (Table 2).

Table 2: WHO Parameters for leaves of *P.zeylanica*

Sl. no.	WHO parameters	Leaves of Catunaregum spinosa
1	Hemolytic activity	MeOH ext.:100mg/ml not shows less hemolysis Water ext.: 8.05
2	Foaming index	Less than 100
3	Total tannin content	1 %
4	Total bacterial count	7 x10^3
5	Total fungal count	6 x10^4
6	*E. Coli*	5 x 10^3
7	Salmonella	Absent

The fluorescence characteristics for the leaf powder with different acids and bases varied from a range of yellow to dark green under the ordinary light whereas for UV light it ranged from yellowish brown to brownish red. The leaf extract also showed a varied range of colors from pale green to greenish brown whereas under UV light it ranged from greenish brown to brownish red (Table 3& 4).

Table 3: Florescence characteristic of leaf powder

Sl. no.	Particulars of the treatment	Under ordinary light	Under UV light (366 nm)		
		P. zeylanica	*C. spinosa*	*P. zeylanica*	*C. spinosa*
1	Powder as such	Light green	Dark green	Greenish red	Brick red
2	Powder + 1N NaOH (aqueous)	Green	Green	Greenish red	Brick red
3	Powder +1N NaOH (alcoholic)	Dark green	Dark green	Brownish red	Reddish green
4	Powder + 1N HCL	Greenish black	Blackish green	Dark brown	Chocolate brown
5	Powder + H_2SO_4 (1:1)	Dark green	Green	Brownish yellow	Brown
6	Powder + HNO3 (1:1)	Greenish yellow	Yellow	Reddish yellow	Orange
7	Powder + Ammonia	Yellow	Greenish yellow	Yellowish brown	Greenish yellow
8	Powder + Iodine	Greenish brown	Dark brown	Dark brown	Brown
9	Powder + 5% FeCl3	Brown	Dark-yellowish brown	Reddish brown	Dark brown
10	Powder + Acetic acid	Green	Light green	Yellowish brown	Orange

Table 4: Florescence characteristic of leaf extracts

Sl. No.	Solvent Extracts	Under ordinary light		Under UV light (366 nm)	
		P. zeylanica	*C. spinosa*	*P. zeylanica*	*C. spinosa*
1	Petroleum ether (40-60°C)	Light green	Green	Greenish Orange	Yellowish green
2	Benzene	Green	Dark green	Brown	Red
3	Chloroform	Green	Dark green	Brown	Red
4	Acetone	Green	Dark green	Brown	Red
5	Methanol	Green	Dark green	Brown	Deep red
6	Ethanol	Green	Dark green	Brownish red	Brown
7	Water	Greenish brown	Brownish green	Brownish red	Blackish brown

The preliminary phyto–profiling for the leaves extracts of *P.zeylanica* was carried out wherein the consistency was found to be sticky in the non polar to not so polar solvent extracts whereas the polar solvent extracts were found to be non-sticky (Table 5). The percentage yield w/w of the extracts was analyzed wherein the highest yield was found to be in the ethanolic extract.

Table 5: Preliminary phyto-profile for leaves of *P. zeylanica*

Sl. No.	*Solvent used*	*Color*	*Consistency*	% Yield w/w
1	Petroleum ether (40-60°C)	Light Green	Non-sticky	1.6
2	Benzene	Green	Sticky	0.4
3	Chloroform	Green	Sticky	0.9
4	Acetone	Green	Sticky	1.19
5	Methanol	Green	Sticky	5.3
6	Ethanol	Green	Sticky	6.05
7	Water	Dark green	Sticky	0.75

P.zeylanica when subjected to phytochemical analysis showed the presence of proteins in ethanolic extract alone and carbohydrates in all the extract. Proteins were present only in ethanolic extract and carbohydrates were present in all the extract. Flavonoids and phenols were present only in ethanolic extract. Alkaloids and terpenoids were present in ethanolic and aqueous extract. Steroids and saponins were absent in all the extracts. Tannins were absent in ethanolic and chloroform extracts (Table 6).

Table 6: Phytochemical analysis of different extracts of *Pavonia zeylanica* leaves

Sl. No.	*Name of the test*	*Procedure*	*Observation*	*P*	*B*	*C*	*A*	*E*	*M*	Aq
1	Alkaloids	Drug + Dragondroffs reagent Mayer's reagent Hager's reagent	Orange color White ppt. Yellow ppt.	– – –	– – –	– – –	– – –	+ + +	+ + +	+ + +
2	Glycosides	Anthrone + H2SO4+ Heat	Purple or green	–	–	–	–	+	–	–
3	Carbohydrates	Drug + Molishs reagent+ conc.H2SO4 Fehling's solution A&B	Purple color Brick red color	– –	– –	– –	– –	– –	– –	+ +
4	Phytosterols/ triterpenoids	Liebermann Test Salkowski Test Noller's test	Bluish green Red & fluorescent Pink color	– – –	– – –	– – –	– – –	+ + +	+ + +	+ + –
5	Proteins & Amino acids	Biuret test Xanthoprotein test Millon's reagent test Lead acetate test Ninhydrin test	Violet color Orange color White ppt White ppt Blue color	– – – – –	– – – – –	– – – – –	– – – – –	+ + + + –	– – – – –	+ + + + +
6	Saponins	Drug + water + shaking	Formation of honey comb like froth	–	–	–	–	–	–	–
7	Flavonoids	Shinodaw's Test Zn-HCl acid reduction Test	Red color Magenta color	– –	– –	– –	– –	+ +	– –	– –
8	Fixed oils & Fats	Spot test	Stains appear after drying	+	–	–	–	–	–	–
9	Gums/Mucilage	Drug + water	No thickening of the substance	–	–	–	–	–	–	–
10	Volatile oil	Spot test	------	+	–	–	–	–	–	+
11	Phenolics/Tannins	Fecl3 Drug + lead acetate + water	Intense color Formation of white ppt	–	–	–	+	+	+	+

*P - Petroleum ether (60-800C); *B - Benzene; *C - Chloroform; *A - Acetone; *M - Methanol; *E - Ethanol; Aq- Aqueous; + - Positive; - Negative

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The presence of phytochemicals in *P.zeylanica* extract revealed that, tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer; similar reports were also made by previous Researchers[11]. Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2. Flavonoids serve as health promoting compound as a results of its anion radicals[12]. These observations support the usefulness of this plant in folklore remedies in the treatment of stress-related ailments and as dressings for wounds normally encountered in circumcision rites, bruises, cuts and sores[13].

Saponins, which are present in plants, have been suggested as possible anti-carcinogens. They possess surface-active characteristics that are due to the amphiphilic nature of their chemical structure. The proposed mechanisms of anti-carcinogenic properties of saponins include direct cytotoxicity, immuno-modulatory effects, bile acid binding and normalization of carcinogen-induced cell proliferation. However, the anti-carcinogenic effects of saponins from commonly consumed plant foods have not been studied. Soybeans are one of the most important sources of dietary saponins. They are the main protein supplier in many vegetarian diets[14].

The plant extract was also positive for steroids which are very important compounds especially due to their relationship with compounds such as sex hormone[15]. The presence of these phenolic compounds in this plant contributed to their anti-oxidative properties and thus the usefulness of these plants in herbal medicament. Phenols have been found to be useful in the preparation of some antimicrobial compounds such as dettol and cresol. This plant is used routinely among many tribes in Africa for the treatment of various diseases.

Alkaloid was not detected in this plant study. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [16], and their absence in this plant tend to lower the risk of poisoning by the plant. Banerjee (2009) in his present investigation has stated the importance of standardization of parameters of *Bridelia retusa bark*, qualitative and quantitative microscopic characters, ash values, extractive values, and phytochemical profiles of petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of the plant. These standardized parameters would be of immense help in authenticating *Bridelia retusa*[17].

Thus, *P.zeylanica* containing these compounds may serve as a potential source of bioactive compounds in the treatment of cancer.

Conclusions

New anticancer drugs derived from research on plant will be continuously discovered. The activities of phytochemicals and the synergistic action shown by them make them ideal in alternative cancer therapies. The chemopreventive effects that most phytochemicals exert are likely to be the sum of their effect on several distinct mechanisms working inside the cell. The phytochemicals have been focused for the research since 1930's but many of them have been used in traditional medicines for thousands of years. Proper identification of drugs and their evaluation on a scientific basis is therefore of prime significance. This can certainly help to rejuvenate the ancient system of medicine like ayurveda.

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