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PHYTOCHEMICAL CONSTITUENTS, PROXIMATE ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF AN ENDEMIC LIANA (ASPIDOPTERYS CANARENSIS)

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Abstract: This study investigated the phytochemical constituents, proximate analysis, antioxidant, antimicrobial properties of methanolic leaf extracts of *Aspidopterys canarensis* Dalz. Results indicated the presence of acidic compounds, phenols, flavonoids, alkaloids, tannins, terpenoids, steroids, carbohydrates and reducing sugar. The proximate analysis showed it has good amount of crude fibre (39.66%) while crude ash (1.0 %) was the least other compounds were protein (0.083 mg/ml), amino acids (0.026mg/ml) and carbohydrate (0.135 mg/ml).The total antioxidant capacity of plant was 60%. The antimicrobial activities of the extracts of *A. canarensis* were studied against five pathogenic bacterial strains and five fungal strains. The antibacterial activity index of *A. canarensis* was found to be highest against *Staphylococcus aureus* and least to *Enterobacter aerogenes*. The maximum antifungal property of the methanol extract of the leaves were found 90.59% inhibition to the growth of *Phytophthora capsici* followed by *Curvularia geniculate* (82.42%) and more than 45% inhibition showed to all the other tested organisms. The results of this study indicate that leaf extracts of *A. canarensis* possess antioxidant, anti-microbial activities which explain its use in animal nutrition and human medicine.

Key Words: *Aspidopterys canarensis*, antimicrobial, antioxidant, endemic, liana, phytochemical constituents, proximate analysis.

INTRODUCTION

Phytochemicals of medicinal plants showing antimicrobial activities have the potential of filling new medicinal need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action are also very likely to differ. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity[1&2]. Screening the active compounds from plants has led to the discovery of new drugs which have efficient protection and treatment roles against various diseases.

A. canarensis is a liana endemic to Western Ghats belongs to the family Malphigiaceae treated as a vulnerable category in Indian Red Data Book[3]. Its natural distribution is restricted in few pockets of semi evergreen forests of Kerala and Karnataka. As part of a species specific recovery program of RET lianas of Western Ghats by MSSRF, this plant undergone mass multiplication and conservation. During this study it was observed that very fragmented information is available on uses or economic importance of this plant. In this back ground this work attempt to screen the phyto-constituents, proximate analysis, antioxidant and antimicrobial properties of this important life form.

MATERIALS AND METHODS

Collection and preparation of sample

The fresh leaves of *A. canarensis* collected from Botanic Garden of M.S. Swaminathan Research Foundation, Kalpetta, Kerala, India. The fresh sample of about 1 kg were collected in polythene bags and taken to the laboratory. The leaves were surface sterilized and washed with clean sterile water. Then the leaves were shade dried until all the water molecules evaporated and dried for one hour at 160°C. After drying the leaves were ground well using mechanical blender into fine powder and then transferred into air tight container.

Preparation of Extract

Plant materials were washed with clean sterile water and oven-dried for one hour at 160°C. 10g of air dried sample was taken in 100ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker for 24 hours. After 24 hours, the supernatant was collected and the solvent was evaporated to make the final volume (one fourth of original volume) and stored at 4°C in air tight bottles.

Phytochemical screening

The different qualitative chemical tests can be performed for establishing a profile of given extract for its chemical composition. The extracts were then subjected to qualitative chemical tests for various phyto-constituents like alkaloids, flavonoids, tannins, phenolic compounds, cardiac glycosides, terpenoids, anthraquinones, saponins and steroids[4-6].

Proximate analysis

Proximate analyses were carried out according to the procedure of Association of Official Analytical Chemist[7&8]. This constitutes the class of food present in samples such as carbohydrate, protein, fat, crude fiber, ash content and moisture content.

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Antibacterial activity

Paper discs impregnated with specific antibiotics for the test substances are placed on the surface of the Nutrient agar medium inoculated with the target organisms, which is recommended for the diffusion of antimicrobial agents as described in NCCLS approved standard. The plates are incubated and the zones of inhibition around each disc were measured.

The overnight culture of pathogenic test organisms swabbed on to Nutrient Agar medium. Different dilutions of five plant extracts were prepared in the order of 0.2g/ml, 0.4g/ml, 0.6g/ml and 0.8g/ml respectively. 10µl of each concentration was introduced into each filter disc (5mm in diameter) and discs were placed in one plate. The plate was incubated at 37°c for 24hours and zone of inhibition was measured. The inhibition zone of plant extracts were compared with antibiotic ampicillin. The activity index of each plant extract was calculated by using the formula.

$$\text{Activity index} = \frac{\text{Inhibition area of sample}}{\text{Inhibition area of standard}}$$

Antifungal Assay

Potato Dextrose Agar medium with different concentrations of 20%, 40% and 60% of the methanolic extracts of the test plants was prepared. About 15 ml of the medium was poured into each petriplate and allowed to solidify. Five mm disc of 7-day-old culture of the test fungi were placed at the center of the petriplates and incubated at 25±2°C for seven days. After incubation the colony diameter was measured in millimeter. For each treatment three replicates were maintained. PDA medium without the methanolic extract served as control. The fungi toxicity of the extracts in terms of percentage inhibition of mycelial growth was calculated by using the formula.

$$\text{Percentage of inhibition} = \frac{dc - dt}{dc} \times 100$$

where dc = Average increase in mycelial growth in control
dt = Average increase in mycelial growth in treatment.

Statistical Analysis

Each assay in this experiment was repeated thrice and the values were expressed as mean of triplicate analysis of the samples (n=3) ± standard deviation (SD).

RESULTS

Phytochemical analysis of the leaf extracts *A. canarensis* revealed the presence of acidic compounds, phenols, flavonoids, alkaloids, tannins, terpenoids, steroids, carbohydrates and reducing (Table 1). Quantitative determination of the phytochemicals present in this plant was also performed (Table 2). The proximate analysis showed crude fiber was the highest (98.4%) while crude ash (1.0%) was the least and also analyzed protein (0.083 mg/ml), amino acids (0.026mg/ml) and carbohydrate (0.135 mg/ml) (Table 3). The leaf of *A. canarensis* was tested for their antioxidant content, their ability to scavenge free radicals and their biomolecular protective effects. The results obtained are presented below in Table 4. The enzymatic antioxidants analysed were catalase and peroxidase. Non-enzymatic antioxidants like NO radical scavenging activity, H_2O_2 scavenging activity and reducing power determination assessed as well.

Table 1: Phytochemical analysis of methanolic extracts of *Aspidopterys canarensis*

Phytochemical	Status
Acidic compounds	+
Phenols	+
Flavanoids	+
Alkaloids	+
Tannins	+
Glycosides	_
Saponins	_
Terpenoids	+
Steroids	+
Antraquinones	_
Carbohydrates	+
Reducing sugar	+
Resins	_

+ = Present; - = absent

Table 2: Quantitative Determination of Phytochemicals in *Aspidopterys canarensis*

Phytochemical	Quantity (mg/ml)
Total phenolic contents	0.033
Flavanoids	0.245
Tannic acid	0.102
Alkaloids	1.800
Total carbohydrates	0.135
Reducing sugar	0.195
Inulin	0.021
Sucrose	0.240
Cellulose	0.030
Ascorbic acid	0.100

Table 3: Proximate analysis result of the blended leaves of *Aspidopterys canarensis*

Plant material	Moisture (%)	Total solids (%)	Crude Ash (%)	Crude fiber (%)	Protein (mg/ml)	Amino acid (mg/ml)	Carbohydrate (mg/ml)
Aspidopterys canarensis	80	20	1	39.66	0.083	0.026	0.135

Table 4: Antioxidant activity of leaves of *Aspidopterys canarensis*

Total antioxidant capacity (%)	Enzymatic antioxidants		Non enzymatic antioxidants		
	Catalase (%)	Peroxidase (%)	NO radical scavenging activity (%)	H_2O_2 Scavenging activity (%)	Reducing power (%)
60	51.99	61.68	9.42	22.72	95

Table 5: Antibacterial activity of methanolic extract of leaves of *Aspidopterys canarensis*

Tested organism	Inhibition zone of Ampicillin in mm	Concentration (in %)	Inhibition Zone (mm)	Activity Index (mm)
Escherichia coli	15.66+0.57	60	6.66+0.57	0.425
Klebsiella pneumoniae	16.33+0.57	40	8.33+0.57	0.51
Staphylococcus aureus	14.66+1.52	40	9.66+1.15	0.658
Enterobacter aerogenes	16+1.73	60	8.33+1.52	0.52
Salmonella typhi	17.33+0.57	40	7.66+1.15	0.45

Mean and standard deviation of three observations

Table 6: Antifungal activity of methanolic extract of leaves of *Aspidopterys canarensis*

Name of organism tested	Colony diameter in mm				% of inhibition at 60%
	Control	20%	40%	60%	
Aspergillus niger	14.66±0.57	8.66±0.57	7.33±1.15	6.66±0.57	54.57
Phytophthora capsici	56.66±1.52	4.33±0.57	4.66±1.52	5.33±1.52	90.59
Fusarium oxysporum	12.66±1.15	5.66±1.52	6.33±1.52	4.33±1.15	65.33
Curvularia geniculata	30.33±0.57	6±1	5.66±1.15	5.33±0.57	82.42
Pythium aphanidermatum	13.33±1.52	4.66±1.52	6.33±1.52	5±1	62.49

Mean and standard deviation of three observations

The antimicrobial activity of the extracts of *A. canarensis* were studied against five pathogenic bacterial strains, namely *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Enterobacter aerogenes* and *Salmonella typhi* and five fungal strains (*Aspergillus niger, Phytophthora capsici, Fusariumo xysporum, Curvularia geniculata, Pythium aphanidermatum*). Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of growth. The antibacterial activity index of *A. canarensis* was found to be highest to against *Staphylococcus aureus* and least to *Enterobacter aerogenes* (Table 5). The fungicidal activities of the leaves of *A. canarensis* are provided in Table 6. The methanolic extract of the leaves were found to cause 90.50% inhibition of the growth of *Phytophthora capsici* followed by 82.42% of inhibition of growth of *Curvularia geniculata* and more than 45% inhibition showed the remaining pathogens.

DISCUSSION AND CONCLUSIONS

Phytochemicals analyzed from the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities[9]. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites[10].

Phenolics content are very important plant constituents because they can act as reducing agents, hydrogen donors and metal chelator[11]. They also act as radical scavenger due to their hydroxyl groups. The total phenolic content of *A. canarensis* was 0.033mg/ml. Natural antioxidants mainly come from plants in the form of phenolic compounds, such as flavonoids, phenolic acids, tocopherolsetc[12]. The anti-oxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation[13]. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall[14]. Flavonoids show their antioxidant action through scavenging or chelating process[15]. More than 2000 flavonoids have been reported among woody and non-woody plants[16]. In this study, the flavanoid content of *A. canarensis* was 0.245mg/ml.

Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity[17] and activity in the nervous system especially in the action towards neurotransmitters such as acetylcoline, epinephrine, norepinephrine, dopamine and serotonin[18]. In the present study *A. canarensis* shows alkaloid content of 1.8mg/ml. It is obvious that this plant can be considered as a resource for the mass extraction of alkaloids for industrial applications. The carbohydrates in food are of major interest in relation to chronic diseases. Different types of carbohydrates give rise to different glycemic responses, and also able to stimulate lipogenesis[19].

Ash in food contributes the residue remaining after all the moisture has been removed as well as the organic material (fat, protein, carbohydrates, vitamins, organic acid) have been incinerated at a temperature

of about 500°C. Ash content is generally taken to be a measure of the mineral content of the original food[8]. Least amount of Ash content was obtained for *A. canarensis* in this study. High percentage of moisture content, this is an indication that they possess large number of cell saps. Water is clearly the most important nutrient and the most abundant substance in the most of the living organisms. The proximate analysis showed the moisture content of *A. canarensis* was 80%. In this work, the selected liana species shows highest amount of crude fiber. Crude fibre in food is an indication of the level of non-digestible carbohydrate and lignin. Crude fibre is made up largely of cellulose together with a little lignin which is indigestible in human[8]. Thus the presence of fibre may have role in providing shape to leaves and imparting health to the plant.

Antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is an increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs, and medicinal plants. The reducing power of bio active compound is generally associated with the presence of reductions which have been shown to exert antioxidant action by breaking the free radical chains by donating a hydrogen atom[20]. The total antioxidant capacity of *A. canarensis* was 60%. Hydrogen peroxide is an important biological reactant because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell[21]. In the present study *A. canarensis* have a moderate source of hydrogen peroxide scavenging activity. Scavenging of H_2O_2 by the plant extracts may be attributed to their phenolics, which donate electron to H_2O_2, thus reducing it to water. The extract was capable of scavenging hydrogen peroxide in a concentration dependent manner.

Nitric oxide (NO) is a reactive free radical produced by phagocytes and endothelial cells, to yield more reactive species such as peroxy nitrite which can be decomposed to form OH radical. The level of nitric oxide in *A. canarensis* was 9.42%. Since NO plays a crucial role in the pathogenesis of inflammation[22] this may explain the use of chosen liana species for the treatment of inflammation and for wound healing. Plants with antioxidant activities have been reported to possess free radical scavenging activity. Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism[11]. Detailed investigations have to be made on the role of nitric oxide from this plant in the healing of wounds and against more clinical pathogens.

The most common ROs molecule is H_2O_2, it is mainly produced by mitochondria as a byproduct of oxidative metabolism. Because a high level of H_2O_2 is cytotoxic and can modify the protein confirmation of alter protein function, the tissue of H_2O_2 scavenging is also important. In addition to ROs, NO is also implicated in several pathological conditions[22]. Plant extracts have been reported to possess components acting as electron donors. The free radicals are produced in aerobic cells due to consumption of oxygen in cell growth. Free radicals cause decrease in membrane fluidity, loss of enzyme receptor activity and damage to membrane protein leading to death[23]. As methanol extract of these plants showed the dose dependent antioxidant activity comparable to ascorbic acid, antioxidant agent might be developed from these plants for the treatment of disorders associated with free radicals. Phenolic compounds containing free hydrogen are largely responsible for antioxidant activity[24], thus the phenol compounds of *A. canarensis* can be referred to be responsible for the antioxidant activity.

The presences of alkaloids and phenolic compounds are thought to be toxic to microorganisms, inhibiting the enzymes which are essential for the growth of microorganisms. Thus there has been a continuing search for new and more potent antibiotics. In this work plant extracts found to be bactericidal and fungicidal properties. Therefore, this work can be an indication for its potential as a drug that can be used against these microorganisms. According to WHO on infectious diseases 2000, overcoming antibiotic resistance is the major issue of the WHO for the next millennium. Hence, the last decade has witnessed an increase in the investigations on plants as a source of human disease management. The results acquired in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and this plants are proving to be a valuable reservoir of bioactive compounds of substantial medicinal merit which we can be commercially exploited.

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