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Mineral Composition, Cytotoxic and Anticariogenic Activity of *Scleropyrum pentandrum* (Dennst.) Mabb

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Abstract

Scleropyrum pentandrum (Dennst.) Mabb belongs to the family Santalaceae and grows along the margin of evergreen to semi-evergreen forests. The present study was designed to investigate mineral composition, cytotoxic and anticariogenic potential of *S. pentandrum* leaves. The mineral content in powdered leaf material was estimated by using atomic absorption spectrophotometer. The methanol extract was tested for anticariogenic activity against 24 oral isolates of *Streptococcus mutans* by Agar well diffusion method. Cytotoxic activity of extract was determined by brine shrimp lethality bioassay against the brine shrimp *Artemia nauplii*. Among the principal elements, high potassium content followed by calcium, magnesium and phosphorus. Among trace elements, iron was detected in high concentration followed by manganese, zinc and copper. The extract was found to cause inhibition of oral bacterial isolates in a dose dependent manner. The diameter of inhibition zone formed was in the range of 1.7 to 2.3cm and 1.3 to 2.0cm at extract concentration of 20mg/ml and 10mg/ml respectively. The lethality of extract against brine shrimps in terms of mortality of shrimps was directly proportional to the concentration of the extract and highest mortality (33%) was observed at 1000µg/ml concentration. Phytochemical screening of extract revealed the presence of alkaloids, saponins, tannins and glycosides and these phytoconstituents could be responsible for the observed anticariogenic and cytotoxic potential of the extract. Further studies on characterization of active components in the extract and their bioactivities are to be carried out.

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Scleropyrum pentandrum, Minerals, Agar well diffusion, *Streptococcus mutans*, Brine shrimp lethality

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INTRODUCTION

Scleropyrum pentandrum (Dennst.) Mabb (Synonym : *Scleropyrum wallichianum* Arn.) belongs to the family Santalaceae and grows along

the margin of evergreen to semi-evergreen forests between 600 and 1600 m. *S. Pentandrun* is distributed in Cambodia, China, Thailand, Sri Lanka and Laos. In India, it is distributed in Peninsular India, Western Ghats, South and Central Sahyadris and generally found on sandy soil, as well found in semi and dry evergreen forests, in open forest near stream and in lowland Dipterocarps forest, from 500 to 1000m. Flowering occurs in January to March, fruiting in August to October [1]. The whole plant parts are applied externally to treat skin irritation in Kani tribal settlement, Agasthiayamalai biosphere reserve, Tirunelveli, South India [2]. It is commonly called Naaikuli in Kasargod, Kerala and is used as a mechanical barrier (fencing) in dried or live condition [3]. The crushed roots are given for curing stomach ailments in Kurichyas tribal community in Kannur district of Kerala [4]. Recently, Soundarya *et al.* [5] screened antibacterial activity methanol extract of leaves and found inhibitory efficacy was dose dependent. Extensive literature reviews revealed that much of the bioactivities of this plant remain unexplored. Hence, the present investigation was undertaken to determine mineral content and to screen anticariogenic and cytotoxic activity of methanol extract of *S. pentandrum* leaves.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Plant material was collected during April 2010 in an area called Kanivebagilu located in Hosanagara Taluk, Shivamogga District, Karnataka. The plant was identified by Prof. KG Bhat, Udupi, Karnataka. Voucher specimen (KU/AB/KSV/3946) was deposited in the University herbaria at PG Department of Studies and Research in Botany, Shankaraghatta, Karnataka for future reference.

Determination of Elemental Composition

Elemental composition was determined according to the method of Sarangi *et al.* [6]. A known amount of powdered leaf material (1gm) was digested using mixture of concentrated nitric acid and perchloric acid (10ml) in a beaker. The powdered material was left in acid mixture for 24 hours and was digested on the hot plate until complete digestion. After digestion, 10ml of 10% nitric acid was added to the beaker and the beaker was left for two hours for residue to settle down. The supernatant liquid was filtered though Whatman No. 1. The filtrate was subjected to estimation of elements namely calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) using Atomic absorption spectrophotometer.

Extraction and Phytochemical Analysis

The leaves were washed thoroughly, shade dried, powdered and used for extraction. A known quantity of powdered leaf material (500gm) was subjected to soxhlation and exhaustively extracted with methanol (HiMedia, Mumbai) for about 48 hours. The extract was filtered, concentrated in vacuum under reduced pressure and dried in the desiccator [7]. Methanol extract was subjected to preliminary phytochemical analysis to screen phytoconstituents namely alkaloids (Dragendorff's test and Mayer's test), saponins (frothing test and hemolysis test), flavonoids (Shinoda test), glycosides (Salkowski test and Keller-Kiliani test), tannins (ferric chloride test) and Terpenoids (Salkowski test) [8,9].

Anticariogenic Activity of Methanol Extract

The anticariogenic efficacy of methanol extract was tested by Agar-well-diffusion method [10] against 24 oral isolates of *S. mutans* (S-1 to S-24) recovered from dental plaque and saliva samples of dental caries patients. The *S. mutans* isolates were maintained on sterile Brain heart infusion agar (HiMedia, Mumbai) slants. Briefly, 24 hours old Brain heart infusion broth (HiMedia, Mumbai) cultures of *S. mutans* isolates were swabbed uniformly on solidified sterile Brain heart infusion agar plates using sterile cotton swab. Then,

wells of 6mm diameter were punched in the inoculated plates with the help of sterile gel puncher and the extract (10 and 20mg/ml of 10% DMSO), Standard (Chloramphenicol, 1mg/ml) and Control (10% DMSO) were added separately into respectively labeled wells. The inoculated plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition formed around the well was measured with a ruler. The experiment was carried in triplicate to get average reading.

Cytotoxic Activity of Methanol Extract

The brine shrimp lethality test was conducted according to the method of Raghavendra *et al.* [11]. Brine shrimp *Artemia nauplii* eggs (Nihon Animal Pharmaceutical Inc., Tokyo, Japan) were hatched in a container filled with air-bubbled artificial sea water which was prepared with 10g of a commercial salt mixture (GEX Inc., Osaka, Japan) and 500ml of distilled water. After 36-48 hours, the phototropic shrimps were collected by pipette for bioassay. The different concentrations of methanol extract (10-1000µg/ml) were tested in vials containing 5ml of brine and 25 shrimp in each of three replicates. The vials were incubated at 25°C and surviving shrimps were counted after 24 hours.

RESULTS

Phytochemical and Mineral Composition

Yield of the extract was 4.17%. Preliminary phytochemical screening of methanol extract revealed the presence of alkaloids, saponins, tannins and glycosides. Mineral content of leaf material is shown in Table 1.

Table 1: Elemental composition of *S. pentandrum* leaves

Element	Quantity (ppm)
P	1600
K	22000
Fe	417.8
Zn	27.5
Mn	209.4
Cu	19.2
Mg	6199.8
Ca	21190.0

Anticariogenic Activity of Methanol Extract

The anticariogenic activity of methanol extract was investigated against 24 oral isolates of *S. mutans* recovered from plaque and saliva samples of dental caries patients. The result of inhibitory activity of extract is shown in Table 2. Results were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth and it as reported as positive and the absence of zone as negative. It was found that the extract caused inhibition of oral bacterial isolates in a dose dependent manner. The diameter of inhibition zone formed was in the range of 1.7 to 2.3cm and 1.3 to 2.0cm at extract concentration of 20mg/ml and 10mg/ml respectively. Inhibition caused by standard antibiotic was higher than that of methanol extract. DMSO did not cause any inhibition of cariogenic isolates.

Table 2: Anticariogenic activity of methanol extract against cariogenic bacteria

Isolate number	Zone of Inhibition in cm			
	Methanol extract		Antibiotic (1mg/ml)	DMSO (10%)
	20mg/ml	10mg/ml		
S-1	1.9	1.8	2.3	-
S-2	1.8	1.7	2.6	-
S-3	1.7	1.5	2.6	-
S-4	1.9	1.8	2.5	-
S-5	1.8	1.7	2.6	-
S-6	2.0	1.8	2.4	-
S-7	2.1	2.0	2.5	-
S-8	1.7	1.6	2.7	-
S-9	2.1	2.0	2.7	-
S-10	1.8	1.7	2.4	-
S-11	1.9	1.7	2.5	-
S-12	2.1	1.9	2.6	-
S-13	2.3	1.9	2.8	-
S-14	1.7	1.5	2.3	-
S-15	1.9	1.3	2.5	-
S-16	1.9	1.5	2.4	-
S-17	2.0	1.7	2.6	-
S-18	1.7	1.6	2.4	-
S-19	1.8	1.6	2.6	-
S-20	1.8	1.5	2.7	-
S-21	2.1	1.9	2.6	-
S-22	1.9	1.6	2.6	-
S-23	1.7	1.5	2.5	-
S-24	2.1	1.9	2.5	-

Brine Shrimp Lethality of Methanol Extract

The result of cytotoxic activity of methanol extract by brine shrimp lethality is presented in Table 3. The lethality of extract in terms of mortality of shrimps was directly proportional to the concentration of the extract. Highest mortality (33%) was observed at 1000µg/ml concentration whereas mortality was not observed at 10µg/ml extract concentration. Thus, the extract was found to be toxic at high doses.

Table 3: Brine shrimp lethality of methanol extract

concentration (µg/ml)	Total number of shrimps	Number of dead shrimps	Mortality (%)
10	100	0	0.0
100	100	7	7.0
1000	100	33	33.0

DISCUSSION

Minerals are chemical constituents used by the body in many ways. Although they yield no energy, they have important roles to play in many activities in the body. These include calcification of bone, blood coagulation, neuromuscular activity, acid base equilibrium, enzyme activity, osmotic regulation etc. Minerals are broadly classified into principal elements and trace elements. Minerals like Ca, K, Mg, P etc forms the principal elements while Fe, Cu, Mn, Zn etc forms the trace elements. K maintains intracellular osmotic pressure and is required for the regulation of acid base balance and water balance in cells. It is required for the transmission of nerve impulse and is necessary for protein synthesis by ribosomes. P is a constituent of high energy phosphate compounds and nucleotide coenzymes. Ca is important for muscle contraction, blood coagulation, nerve transmission, membrane integrity and permeability, activation of certain enzymes, development of bones and teeth. Mg is required for the formation of bones and teeth and is necessary for proper neuromuscular function. It serves as a cofactor for several enzymes requiring ATP. Fe is an important component of hemoglobin and myoglobin. It is present in cytochromes and certain non-heme proteins that are involved in electron transport chain and oxidative phosphorylation. Iron is associated with effective immunocompetence of the body. Cu is an essential component of several enzymes and is necessary for the synthesis of hemoglobin, melanin and phospholipids. Cu is important for the development of bone and nervous system. Mn is required for the formation of bone, synthesis of hemoglobin, cholesterol and normal functioning of nervous system. It serves as cofactor for several enzymes and it inhibits lipid peroxidation. Zn is important in storage and secretion of insulin from pancreas. It is an essential component of several enzymes and is required for wound healing [12,13]. In the present investigation, appreciable quantities of most of these minerals were observed in the leaf material. Among the principal elements, potassium is present in high concentration (22000 ppm) followed by calcium (21190 ppm), magnesium (6199.8 ppm), and phosphorus (1600 ppm). Among trace elements, high concentration of iron is detected (417.8 ppm) followed by manganese (209.4 ppm), zinc (27.5 ppm) and copper (19.2 ppm).

Dental caries is the most common infectious diseases in the oral cavity. Approximately 200 to 300 bacterial species colonize human dental plaques, but as noted above only a finite number have been associated with either dental caries or periodontal disease [14]. Among the oral bacteria, mutans streptococci have been considered as the major cariogenic bacteria. Mutans streptococci are further divided into seven species namely *Streptococcus mutans*, *S. sobrinus*, *S. downei*, *S. rattus*, *S. cricetus*, *S. ferus*, and *S. macacae*. Among these, *S. mutans* is the major agent of this disease in man, followed by *S. sobrinus*, which has also been implicated in this process. Other species such as *S. cricetus* and *S. rattus* are less frequently isolated from humans as

they are mainly related to dental caries in animals. *S. ferus* does not seem to be related to the etiology of dental caries [14,15-18]. Several studies have been carried on the inhibitory role of natural compounds/extracts against mutans streptococci. Yanagida *et al.* [19] investigated and found the inhibitory effects of apple polyphenols on the synthesis of water-insoluble glucans by glucosyltransferases of streptococci of the mutans group and on the sucrose-dependent adherence of the bacterial cells. The inhibitory activity of methanol extract of *Rheum undulatum* root against *S. mutans* and *S. sorbinus* was investigated by Song *et al.* [20]. The dichloromethane fraction showed the most active antibacterial activity. The activity of the fraction was related to the presence of anthraquinones, cardiac glycosides, coumarines, sterols/terpenes, and phenolics. Esmaeelin *et al.* [21] investigated anticariogenic effect of ethanol and chloroform extracts of *Alcea longipedicellata* against *S. mutans*, *S. salivarious*, *S. sorbinus* and *S. sanguis*. Both the extract were found to be bacteriostatic while malvidin-3,5-diglucoside, isolated from ethanol extract of flowers was found to be the principal constituent for antibacterial activity. In a study, Zheng *et al.* [22] observed inhibitory efficacy of methanol extract of *Aceriphyllum rossii* Engler root and its components aceriphyllic acid A and 3-oxoolean-12-en-27-oic acid against all cariogenic bacteria tested. Aceriphyllic acid A was found to possess faster bacteriostatic activity and the inhibitory action was shown to be membrane disruption leading to killing of bacteria. In our study, concentration dependent inhibition of cariogenic isolates was observed. The leaf extract was found to contain secondary metabolites namely alkaloids, saponins, tannins and glycosides. Antibacterial activity of these secondary metabolites from plant extracts has been well documented and the inhibitory efficacy of extract against cariogenic isolates was linked to the presence of these metabolites by several researchers. In this study also, the inhibitory activity of leaf extract could be related to the phytoconstituents.

The brine shrimp lethality bioassay is considered to be useful in determining various biological activities of extracts such as cytotoxic, phototoxic, pesticidal, trypanocidal, enzyme inhibition, and ion regulation activities [23-28]. This bioassay is a rapid, inexpensive and simple for testing efficacy of plant extracts which in most cases correlates reasonably well with cytotoxic and anti-tumor properties. The assay is based on the ability of the plant extract to cause mortality of laboratory cultured *Artemia nauplii* brine shrimp. It is considered as useful tool for preliminary assessment of toxicity. This is a rapid method utilizing only 24 hours, inexpensive and needs no special equipment. It is so simple that no aseptic technique is required. It utilizes a large number of organisms for validation and a relatively small amount of sample. It does not require animal serum as needed for other methods of cytotoxicity testing [29-31]. Several studies have been carried on brine shrimp lethality of extracts from natural sources. Raghavendra *et al.* [11] showed cytotoxic activity of methanol extract of *Putranjiva roxburghii* Wall (Euphorbiaceae) seeds. The extract was found to be toxic with LC_{50} of 427.74 µg/ml. The cytotoxicity of methanol extract of leaves of *Abrus pulchellus* Wall (Fabaceae) using brine shrimp lethality bioassay revealed dose dependent activity with LC_{50} of 281.70µg/ml [32]. In this study, the extract was found to cause mortality of brine shrimps in a dose dependent manner. The methanol extract showed toxicity only at high dose.

CONCLUSION

In this study, an appreciable quantity of minerals was detected and hence, the plant could be used as a source of important minerals. The extract exhibited anticariogenic and brine shrimp lethality and the

efficacy could be due to the presence of secondary metabolites. The plant could be used as a source of important elements needed for normal physiology of the body. In suitable form, the extract could be used against dental caries and cancer or tumor. Further study is needed to isolate and characterize the bioactive components present in the extract and their anticariogenic and cytotoxic efficacy.

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REFERENCES

1) Ramasubbu R and Chandra Prabha A. A note on floral biology of *Scleropyrum pentandrum* (Dennst.) Mabb. Journal of Biosciences Research 2010; 1(1): 37-39.
2) DeBritto AJ and Mahesh R. Evolutionary medicine of Kani tribal's botanical knowledge in Agasthiayamalai biosphere reserve, South India. Ethnobotanical Leaflets 2007; 11: 280-290.
3) Prasad SK and Raveendran K. Traditional plant fencing and its conservatory nature in Kasaragod district, Kerala, India. Ethnobotanical Leaflets 2010; 14: 681- 686.
4) Rajith NP and Ramachandran VS. Ethnomedicines of Kurichyas, Kannur district, Western ghats, Kerala. Indian Journal of Natural Products and Resources 2010; 1(2): 249-253.
5) Soundarya S, Banakar S, Thippeswamy B, Thippeswamy NB, Kekuda TRP, Mukunda S and Vinayaka KS. Phytochemical screening and Antibacterial activity of leaf extract of *Scleropyrum pentandrum* (Dennst.) Mabb (Santalaceae). Research and Reviews in Biomedicine and Biotechnology 2011; 2(1&2): 40-43.
6) Sarangi RK, Kathiresan K and Subramanian AN. Metal concentrations in five mangrove species of Bhitarkanika, Orissa, east coast of India. Indian Journal of Marine Sciences 2002; 31(3): 251-253.
7) Manjunatha BK, Patil HSR, Vidya SM, Kekuda TRP, Mukunda S and Divakar R. Studies on the antibacterial activity of *Mucuna monosperma* DC. Indian Drugs. 2006; 43: 150-152.
8) Mallikarjuna PB, Rajanna LN, Seetharam YN and Sharanabasappa GK. Phytochemical studies of *Strychnos potatorum* L.f.- A medicinal plant. E-Jour Chem 2007; 4(4): 510-518.
9) George NJ, Obot JB, Ikot AN, Akpan AE and Obi-Egbedi NO. Phytochemical and Antimicrobial properties of leaves of *Alchonea cordifolia*. E-Jour Chem 2010; 7(3): 1071-1079.
10) Vinayaka KS, Swarnalatha SP, Preethi HR, Surabhi KS, Kekuda TRP and Sudharshan SJ. Studies on *In vitro* Antioxidant, Antibacterial and Insecticidal Activity of Methanolic Extract of *Abrus pulchellus* Wall (Fabaceae). African Journal of Basic and Applied Sciences. 2009; 1 (5-6): 110-116.
11) Raghavendra HL, Kekuda TRP, Valleesha NC, Sudharshan SJ and Chinmaya A. Screening for Cytotoxic activity of Methanol Extract of *Putranjiva roxburghii* Wall (Euphorbiaceae) Seeds. Phcog J 2010; 2(10): 335-337.
12) Satyanarayana U. Biochemistry. Books and Allied (P) Ltd., Kolkata, India, 2005, pp 449-465.
13) Soetan KO, Olaiya CO and Oyewole. The importance of mineral elements for humans, domestic animals and plants: A review. African Journal of Food Science 2010; 4(5): 200-222.
14) Loesche WJ. The identification of bacteria associated with periodontal disease and dental caries by enzymatic methods. Oral Microbiol Immunol. 1986: 1: 65-70.
15) Kristofersson K, Grondahl JG and Bratthall D. The more *Streptococcus mutans*, the more caries on approximal surfaces. J Dent Res. 1985; 64: 58-61.
16) Höfling JF, Spolidório DMP, Pereira CV, Rosa EAR and Moreira D. Presença de *Streptococcus mutans* associado a *Streptococcus sobrinus* em escolares de diferentes classes sócio-econômicas esua relação

com a atividade cariogênica dessas populações. Rev Odontol Univ São Paulo. 1999; 13: 173-80.

17) Balakrishnan M, Simmonds RS and Taggs JR. Dental caries is a preventable infectious disease. Aust Dent J. 2000; 45: 235-45.

18) Amoroso P, deAvila FA and Gagliardi CMO. Prevalence of different *streptococci* species in the oral cavity of children and adolescents. Braz J Oral Sci. 2003; 2(4): 164-168.

19) Yanagida A, Kanda T, Tanabe M, Matsudaira F and Cordeiro JGO. Inhibitory effects of Apple Polyphenols and Related Compounds on Cariogenic Factors of Mutans Streptococci. J Agric Food Chem 2000; 48(11): 5666-5671.

20) Song JH, Yang TC, Chang KW, Han SK, Yi HK and Jeon JG. In vitro anti-cariogenic activity of dichloromethane fraction from *Rheum undulatum* L. root. Arch Pharm Res. 2006; 29(6): 490-496.

21) Esmaeelian B, Kamrani YY, Amoozegar MA, Rahmani S, Rahimi M and Amanlou M. Anti-cariogenic properties of malvidin-3,5-diglucoside isolated from *Alcea longipedicellata* against oral bacteria. Int. J. Pharmacol. 2007; 3: 468-474.

22) Zheng CJ, Oh HW and Kim WG. Potent anticariogenic activity of *Aceriphyllum rossii* and its components, aceriphyllic acid A and 3-oxoolean-12-en-27-oic acid. J. Food Sci. 2010; 75(2): 78-82.

23) Jerry L, McLaughlin, Lingling Rogers L. The use of biological assays to evaluate botanicals. Drug Information Journal 1998; 32: 513-524.

24) Anderson JE, Goetz CM, McLaughlin JL, Suffness M. A blind comparison of simple bench-top bioassays and human tumor cell cytotoxicities as antitumor prescreens. Phytochem Anal. 1991; 2: 107-111.

25) Fatope MO, Ibrahim H, Takeda Y. Screening of higher plants reputed as pesticides using the brine shrimp lethality assay. International Journal of Pharmacognosy 1991; 31: 250-254.

26) Solis PN, Wright CW, Anderson MM, Gupta MP, Phillipson JD. A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). Planta Med. 1993; 59: 250-252.

27) Zani CL, Chaves PPG, Queiroz R, Oliveira AB, de Cardoso JE, Anjos AMG, Grandi TSM, Brine shrimp lethality assay as a prescreening system for anti-*Trypanosoma cruzi* activity. Phytomed. 1995; 2: 47-50.

28) Ozala T, Vuorela P, Kivranta J, Vuorela H, Hiltunen R. A bioassay using *Artemia salina* for detecting phototoxicity of plant coumarins. Planta Med. 1999; 65: 715-718.

29) Hossain AM, Ferdous T, Salehuddin SM and Das AK. *In vitro* cytotoxicity (LC_{50}) of extracts obtained from the seeds of *Zea mays*. Asian Journal of Food and Agro-Industry 2009; 2(3): 336-34.

30) McLaughlin JL. Methods in plant biochemistry. In; Hostettmann K., (Ed) Assay for bioactivity. Academic press. London, 1991; 1-32.

31) Luis CJ, Zairo LH, Pillar P and Naira DG. A comparison between two brine shrimp assay to detect in vitro cytotoxicity in marine natural products. BMC Biotechnology 2002; 2(17): 1-5.

32) Kekuda TRP, Raghavendra HL, Surabhi KS, Preethi HR and Swarnalatha SP. Cytotoxic activity of methanol extract of *Abrus pulchellus* Wall (Fabaceae) leaves. Biomedicine 2010; 30(3): 377-379.