WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 3, 747-754. Research Article ISSN 2277– 7105

PROXIMATE COMPOSITION AND PHYTOCHEMICAL ANALYSIS OF SOLVENT EXTRACT OF *THUIDIUM TAMARISCELLUM* (C.MUELL.)BOSCH. & SANDE-LAC. A MOSS.

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Article Received on
22 Dec 2015,

Revised on 13 Jan 2016,
Accepted on 03 Feb 2016

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ABSTRACT

Bryophytes are poikilohydric oldest terrestrial species. The emerging multiresistant pathogens proved to be serious threat to human health globally. Treatment with classical medicine is ineffective in most cases due to different resistant mechanisms hence the need for cheap, effective and natural herbal compounds is need of the hour. This study reported the phytochemical profiling of *Thuidium tamariscellum* a cosmopolitan moss species available along the hill ranges of Kerala. As an initial part of the study, a thorough qualitative analysis was attempted using various solvent extracts. It revealed the presence of alkaloids, flavonoids, glycosides, saponins, triterpenoids, coumarins, tannins and phlobatannins. Further the major phytochemicals were quantified. Terpenoids represented in remarkable levels compared to other secondary metabolites. Further studies are warranted to fractionate, purify the lead molecule and to analyze its biological properties.

KEYWORDS: Bryophytes, solvent extracts, terpenoids, medicinal.

INTRODUCTION

The bryophytes comprise three major lineages such as liverworts, mosses and hornworts. They comprise the oldest known land plants with 22,000 species distributed all over the world. Although Bryophytes are cosmopolitan, their therapeutical importance is not fully exploited wholly. They are employed in pharmaceutical products, horticulture, household decorations and are also ecological indicators of environmental conditions.[1, 2] *Polytrichum* and *Fissidens* species were used as diuretic and hair growth initiating drugs in China.[1, 3]

Moreover, North American Indians used *Polytrichum juniperinum*, *Bryum*, *Mnium* and *Philonotis* mosses to heal wounds, bruises and burns. [1] They also exhibit microbicidal effects against fungi and bacteria (Vats et al., 2012; Alam, 2012). *Marchantia tosana* exhibited fungicidal, bactericidal and antimetastatic activity (*Lahlou et al., 2000*). Bryophytes are not damaged by fungi, bacteria, larvae because of its phenylquinone, aromatic and phenolic compounds which provide defense. [1, 3] Of these, the liverworts possess cellular oil body which produce diverse mono-, sesqui- and di-terpenoids, aromatic compounds like bibenzyl, bis-bibenzyls and acetogenins and are enantiomers of those present in flowering plants. Most of these molecules display unique odour, with potential biological activities. More than 700 terpenoids and 220 aromatic compounds isolated from bryophytes have been evaluated for their biological activities. Various compounds isolated display high potential as chemotherapeutic agents. It has been reported that marchantin A, isoriccardin C, riccardin B, plagiochin E, and marchantin C isolated from *Reboulia hemisphaerica* were found to show cytotoxicity against EYFP-tubulin HeLa cells.[4] Marchantin A which was isolated from *Marchantia emarginata* subsp. tosana was found to induce apoptosis in MCF-7 cells.[1] A macrocyclic bisbibenzyl, riccardin D, which was isolated from *Dumortiera hirsuta* inhibited the proliferation of human non-small-cell lung cancer (NSCLC) both *in vitro* and *in vivo* and does not cause toxicity in normal mammalian cells. However, researchers like biologists, chemists and pharmacologists have been keenly interested in these amphibious plants. The phytochemistry of bryophytes was difficult for a long time because they are small size and difficult to identify, collect in large quantities as pure samples. So far, the chemical compounds and biological activities of *Thuidium tamariscellum* have not been reported in literature. Thus, this study was carried out to determine the phytochemical constituents in this moss.

MATERIALS AND METHODS

Plant material

Thuidium tamariscellum is a moss belonging to Thuidiaceae. Plant body is yellowish-green forming mats, filamentous in appearance, main stem creeping, distributed in wide range of habitats such as rocks, base of trunks and leaf litter. The identity is confirmed by floras and authenticated by comparing with the herbarium of University of Calicut.

Hot continuous soxhlet extraction

Fresh plants were chopped, air dried and sequentially extracted with 250 ml of chloroform, petroleum ether, ethyl acetate, ethanol and water for 8 h continuous hot extraction by soxhlet method.

Preliminary qualitative phytochemical analysis

The different solvent extracts from soxhlet hot continuation method were evaluated for the presence of secondary metabolites according to the protocol of Khandelwal.[5]

Estimation of flavonoids

The total flavonoid content was determined by $AlCl_3$ method with slight modification.[6]

Estimation of alkaloids

Alkaloids in the samples were precipitated using drangendorff reagent and were allowed to stand for 10 min. Later on samples were centrifuged and precipitate was dissolved in Conc.HNO_3. Yellow colour was formed to above solution using 3% thiourea solution and absorbance was read at 435 nm.

Estimation of terpenoids

Total terpenoids was determined by the method of Ferguson.[7]

Determination of saponins

The total saponin content in the plant was determined by the method of Obadomi.[8]

Estimation of soluble proteins

The soluble proteins were estimated by using the method of Lowry.[9]

Estimation of sugars

Sugar content of leaves was estimated by the method of Miller.[10]

Estimation of total phenols

Total phenol content of leaves was estimated by the method of Mayr.[11]

Total Carotenoids

Carotenoid content was estimated by the method of Vicas.[12]

RESULT AND DISCUSSIONS

Phytochemical analysis revealed the presence of pool of secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, triterpenoids, coumarins, tannins and phlobatannins which are considered as active medicinal phytochemical constituents (Table-1). Chloroform extract contain flavonoids, triterpenoids, reducing sugar and tannins. Meanwhile, petroleum ether extract showed alkaloids and glycosides only. Aqueous extract contain saponins, coumarins and Phlobatannins.

Table-1: Phytochemical screening of *T. tamariscellum* using various solvent extracts.

Secondary metabolites	Chloroform	Petroleum ether	Ethyl acetate	Ethanol (90%)	Water
Alkaloids	_	+++	_	+	-
Flavonoids	+++	_	_	_	+
Glycosides	_	+++	_	_	_
Saponins	_	_	_	_	+++
Triterpenoids	+++	_	++	+	_
Reducing sugar	+++	_	_	_	+
Coumarins	_	_	_	_	++
Phlobatannins	_	_	_	_	+++
Tannins	++	_	+	_	_
Anthraquinones	_	_	_	_	_

Subsequently, the major phytochemicals of *Thuidium tamaricellum* were quantified. Alkaloids, terpenoids, flavonoids, saponins and phenols revealed substantial levels (Table 2) and that are reported of having many pharmacological activities confirming that the moss possess medicinal properties. The moss *Thuidium tamariscellum* also showed remarkable level of proteins, sugars and carotenoids (Table 2) suggesting its importance as nutrient and antioxidant feature.

Table-2: Proximate composition of *T. tamariscellum*.

Compound	Concentration (mg/g)
Carbohydrate	3.53 ±0.05
Protein	9.7 ±0.1
Alkaloid	3.4 ±0.22
Phenol	3.2±0.04
Carotenoids	0.22± 0.001
Flavonoid	4.83±0.06
Saponins	2.2±0.03
Terpenoids	25.95± 2.2

Liverworts (Marchantiophyta) possess terpenoids, acetogenins, and aromatic compounds including flavonoids with more than 40 new carbon skeletons. Some of the isolated compounds from liverworts show antimicrobial, antifungal, antiviral, allergenic contact dermatitis, cytotoxicity, insect antifeedant and mortality, antioxidant, nitric oxide (NO) production and plant growth inhibitory, neurotrophic and piscicidal activity, tublin polymerization inhibitory, muscle relaxing, and liver X-receptor (LXR)α agonist and (LXR)β antagonist activities.[13]

Nebojsa et al[14] the presence of flavonoids (aglycones and glycosides), phenolic acids and triterpenes in three bryophyte species: *Polytrichum formosum, Eurhynchium hians* (mosses) and *Pellia endiviifolia* (liverwort). · Adedeji et al[15] analyzed the two moss species *Thuidium gratum* and *Barbula indica* in terms of flavonoids, saponin and alkaloids and the saponins and compared with the medicinal plants of Nigeria and suggested that these two mosses are potential source of useful drugs and can be used for the treatment of many ailments. Saponins have properties of precipitating and coagulating red blood cells and they also have cholesterol binding properties, formation of foams in aqueous solutions and hemolytic activity.

Flavonoids are water soluble phytochemicals and important plant phenolics. They have anti cancer, anti inflammatory activities and a large effect in lower intestinal tract and heart disease. Adriana Basile[16] isolated and identified seven pure flavonoids from five mosses (*Bartramia pomiformis, D. scoparium, P.aane, P. cuspidatum, Hedwigia ciliate*). The flavonoids were the flavones apigenin, apigenin-7-*O*-triglycoside, lucenin-2, luteolin-7-*O*-neohesperidoside, saponarine and vitexin; and the biflavonoid bartramiaflavone and reported of having potential antibacterial activities. Remya Krishnan[17] reported and isolated flavonoids from cell suspension culture from *Marchantia linearis*. The flavonoid type synthesized by mosses also includes o-glyc, c-glyc, flavonols, biflavones,aurons, isoflavons, dihydroflavon, macrocyclic biflavonoids and 3-deoxyanthocyanins.[18] Seven flavonoids were isolated from *Hylocomium splendens*. The majority of the compounds reported in the bryophytes are lipophilic terpenoids (mono-, sesqui-, and diterpenoids) and fragrant compounds. Few of them are nitrogen- or sulfur-containing compounds.[19, 20] Terpenoids usually found in mosses are diterpenoids and triterpenoids. Sabovljević Marko[18] reported the presence of various terpenoids in bryophytes like *Conocephalum conicum* (α-pinene, camphen, sabinene, myrcene, α- and γ-terpinene, limonene, p-cymene terpinen-4-ol, lineoyl acetate, bornyl acetate, α-terpineol), *Frullania tamarisci* (α- and β-pinene, camphen)

Jungermannia cordifolia (α-pinene, camphen, myrcene, α-terpinene, limonene), *Porella platyphylla* (α-pinene, camphen, sabinene, α-terpinene, limonene, p-cymene, β- phellandrene, terpinolene), *Radula complanata* (α -and β-pinene, camphene), *Thamnium alopecurum* (22(29)-hopene), *Thuidium tamarisci* (22(29)-hopene, 7-fernene, 9(11)-fernene, 21-hopene). The sesquiterpenoids costunolide and tulipinolide from *Conocephalum supradecompositum*, *Frullania monocera*, *F. tamarisci*, *Marchantia polymorpha*, *Porella japonica*, and *Wiesnerella denudate* are reported to have anticancer activities.[21, 22] The most anticancer activity was found in Brachytheciaceae, Dicranaceae, Grimmiaceae, Hypnaceae, Mniaceae, Neckeraceae, Polytrichaceae, and Thuidiaceae.[23] *Eurhynchium angustirete*, *Rhytidiadelphus squarrosus* and *Rhodobryum roseum*, and liverwort species, *Frullania dilatata* and *Lophocolea heterophylla* were proven for fungicidal activitied because of their polyphenolic.[24] Asakawa[25] reported antitumour and antiviral activities of bryophytes based on terpenoids in the species. Alam[26] also reported the alcoholic extracts of *Entodon nepalensis* against some pathogenic bacteria. Their striking resistance to microbial infections suggests their inherent synthesis of bactericidal compounds. In addition, *Calymperes erosum* and *Bryum coronatum* display potential bactericidal properties.[27] Recently, Abu Bakar.[28] proved antioxidant and antiproliferative properties *Lepidozia borneensis*,

CONCLUSION

Thuidium tamariscellum is one of the moss which was used in traditional medicine. The crude extract in various solvents showed a pool of phytochemicals and therefore continues to play an essential role in local health care. Further, there is a continuous and urgent need to discover lead molecule with chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and emerging infectious diseases.

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