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Review Article

STUDY ON PHYTOCHEMICAL SCREENING FROM CLADODE EXTRACTS OF *CASUARINA EQUISETIFOLIA*. L., USING VARIOUS POLAR SOLVENTS

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ABSTRACT

The current study has been done to screen the active secondary metabolites present in the cladode extract of *Casuarina equisetifolia*.L., using different polar solvents. The plant sample was collected from Nemilancherry (Pudhucherry district, Tamilnadu) and was authenticated by Botanical Survey of India. The dried and powdered cladode samples were extracted using water, ethanol (9:1) and methanol (9:1). The resulting filtrate was concentrated using rotary evaporator and was used for comparativequalitative screening of potential phytochemicals present in the extract of various solvents, using standard procedure. The results obtained indicates that ethanol cladode extract of *Casuarina equisetifolia*.L., serves as a suitable solvent , that could be used for the extraction of active phytochemicals. Thus this extract could have its application both in food and pharmaceutical industry.

INTRODUCTION

Casuarina is a forest crop known for its high calorific value. It is also widely used as a raw material in paper industry for its efficient cellulose content (Grey *et al*., 2010). It has a great potential to grow under any stress condition like high salinity, alkalinity, anaerobic soil and sterile soil (Tewari, 1994).This species belong to the family Casuarinaceae. The roots of these species are in symbiotic association with *Frankia,*ecto-mycorhizalfungi andendo- mycorhizal fungi, which gives them the potential to survey in the soil that lack nitrogen and phosphorous (Balasubramanian, 2001). The phytochemicals present in the various parts of this forest crop that could be pharmacologically utilized, are less explored. Plants with such phytochemical efficacy serves as source food supplement, folk medicine, pharmaceutical intermediates and also as chemical precursor for synthetic drugs (Ncube *et al*., 2008). Extraction of the plant material for the phytochemical screening is based on the solvent. Solvents play a major role in the analysis of the phytochemicals present in the extract, because it has great impact in rate of extraction, compounds to be extracted, toxicity of the solvent could also affect the bioassay process (Eloff, 1998).

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Mechanism action of the phytochemicals differs from each other. Phenols have antimicrobial and anthelmintic property (Cowan,1999), flavonoids are known for its antidiarrheal property, tannin possess antimicrobial, anthelmintic (Sharma *et al*., 2010), antidiarrheal activity, saponins are proves to possess anticancer property. Thus the current study aims the preliminary screening of phytochemicals using three polar solvents viz. water, methanol, ethanol from the cladode extract of *Casuarinaequisetifolia*.L., This could serve as attempt to elucidate the potential polar solvent that could be used in the extraction process and also its further usage as a new source for therapeutic and industrially useful compound, with medical significance.

MATERIALS AND METHODS

Plant material collection

The cladode samples from *Casuarinaequisetifolia*.L., an exotic tree species were collected from Nemilancherry village, in Pudhucherry Union Territory , Tamilnadu (Latitude: 12.10·, Longitude 79.9·).

Authentication of plant specimen

The pressed dried plant specimen was prepared and sent to BSI (Botanical Survey of India) for authentication of genus and species of plant specimen.

Preparation of sample

The collected cladode samples were washed thoroughly in running water. The samples were cut into small pieces and were shade dried. The dried samples were grinded in mortar and pestle.

Extraction of compounds

The powdered cladode sample was extracted with 3 different solvent viz. water, ethanol and methanol.

Condition for extraction

- 10g of the powdered cladode sample was taken in a 1000ml flask, distilled water was added and was kept in the shaker for 24 hours.
- 10g of the powdered cladode sample was taken in 1000ml flask to which ethanol and water was added in the ratio 9:1. The mixture was macerated and kept in the shaker for 24 hours.
- 10g of the powdered cladode sample was taken in 1000ml flask. Methanol and water was added in the ratio 9:1 and the macerated mixture was kept in the shaker for 24 hours.

The solvent mixture was filtered using Whatman No:1 filter paper.The filtrate was evaporated using rotary vacuumevaporator, in a water bath.The residue of all the three solvents were collected individually, which was used for the qualitative analysis of the potential phytochemicals present in them.

Qualitative phytochemical analysis

Alkaloids (Dragendroff's test)

To 1ml of the extract few drops of dragendroff's reagent was added. The formation of orange coloured precipitate shows the presence of alkaloids (Sofowora, 1993).

Carbohydrates (Molisch's test)

To a 1ml of the extract, few drops of Molisch's reagent was added, followed by the addition of conc. Sulphuric acid along the sides of the test tube. The mixture was then allowed to stand for 2 minute. Then it was diluted with 5ml of distilled water. Formation of red or dull violet colour at the interphase of two layers indicates the presence of carbohydrates (RafiqKhan *et al*., 2013).

Cardiac Glycosides (Keller Killiani Test)

To 1ml of extract, 1ml of Glacial acetic acid and few drops of 2% Ferric chloride was added and then 1ml of conc. Sulphuric acid. Appearance of Brown ring shows presence of Cardiac glycosides (Edeoga *et al*., 2005).

Flavonoids (Sodium hydroxide test)

About 1ml of extract was dissolved in water and filtered, to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids (Trease and Evans., 2002).

Glycosides (Sodium hydroxide reagent)

Dissolve a small amount of extract in 1 ml of water and then add sodium hydroxide solution to the dissolved extract. Formation of yellow colour indicates the presence of glycosides.

Phenols (Ferric chloride test)

5% Ferric chloride was added to 2ml of the extract. Formation of deep blue or black colour will indicate the presence of phenols (Mace, 1963).

Protein and Aminoacid (Ninhydrin test)

The Ninhydrin test is used to detect the presence of alpha-amino acids and proteins containing free amino groups. 2ml of extract was boiled with few drops of 0.2% ninhydrin, appearance of Blue colour indicates the presence of protein.

Phytosterol (Salkowski reaction)

Small quantities of various extracts were dissolved in 5ml of Chloroform separately. This solution was subjected to detect phytosterols. To 0.5 ml of chloroform extract in a test tube add 1ml of Conc. H_2SO_4 from the sides of the test tubes. Appearance of reddish brown colour in chloroform layer indicates presence of phytosterols.

Saponins (Foam test)

The extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously for 15minutes. The formation of stable foam was taken as an indication for the presence of saponins (Sofowora,1993).

Table 1. Qualitative phytochemical analysis of aqueous, methanol and ethanol cladode extract

S.no	Phytochemical test	Aqueous	Methanol	Ethanol
1	Alkaloids	-	-	+
2	Carbohydrate	+	+	+
3	Cardiac Glycosides	++	++	++
4	Flavnoids	-	-	+
5	Glycosides	-	+	+
6	Phenols	++	+	+
7	Protien and Amino Acids	-	-	-
8	Phytosterol	+	-	-
9	Saponin	++	+	++
10	Tanins	+	+	+

Tannin

About 0.5ml of extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution (Preparation: 0.01g of $FeCl_3$ was added to 10ml of distilled water) were added to 2 ml of the filtrate occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins (Trease and Evans, 2002).

RESULTS

Authentication of plant specimen

The plant material was identified taxonomically as *Casuarinaequisetifolia* L. – Casuarinaceae and authenticated with batch number BSI/SRC/5/23/2015/Tech/2012 by Botanical Survey of India.

Qualitative phytochemical analysis

The results of the phytochemical analysis clearly indicate the presence of wide range of potential bioactive compounds in various polar solvents. The qualitative phytochemical analysis shows the presence of alkaloids, carbohydrate, cardiac glycosides, flavonoids, glycosides, phenols, phytosterol, saponin and tannin in appreciable amount (table:1) in all the three polar solvent cladode extracts viz. aqueous, ethanol and methanol. Phenol is a white crystalline aromatic, organic compound which is of high commercial importance found abundantly in aqueous cladode extract. Higher concentration of cardiac glycosides and saponin was found in the aqueous cladode extract. Proteinare composed of amino acid which an organic compound, were not found in any of the three polar solvent. Cardiac glycoside, is an organic compound with glycoside that has its major role in contractile force of cardiac muscle and saponin, a secondary metabolitewith foaming characteristic when consumed lowers the risk of cholesterol in addition to hemolyticproperty, were predominantly found excessive in aqueous, ethanol and methanol extracts of cladode. Carbohydrate, is a polysaccharidebiomolecule, which plays a key role in development of immune system, blood clotting and tannin, is a polyphenolic compoundwhich was present in moderate amount in aqueous, ethanol and methanol extracts of cladode. Presence of alkaloid (organic compound with nitrogen containing bases that has diverse medicinal properties)and Flavonoid (Phenolic compound that was extracted using polar solvents) was observed only in ethanolic cladode extract in moderate quantity. The bonding of sugar with the other functional group via glycosidic bond results in the formation of glycosidic molecule. Moderate presence of glycosides was observed in both the alcoholic cladode extract. Phytosterolis an exclusive plant steroid that sis similar to cholesterol, was found in aqueous extract and was totally absent in methanol and ethanol extract of cladode.

DISCUSSION

The phytochemical screening of cladode extract of *Casuarinaequisetifolia,* through HPLC analysis, revealed the presence of 8 phenolic compounds, gallic, protocatioic, chlorogenic, p.hydroxybenzoie, p.coumaric, syringic, vanillic and salicylic acid (Nehad *et a*l., 2012). A study wasconducted by Harisaranraj *et al.* (2010) in the cladode extract of *Casuarinaequisetifolia,*reported the presence of alkaloids, flavonoids, phenols, tannins, steroids, glycosides and terpenoids..Similar study was also conducted by Iqbal and Arina (2001), who stated that tannin and phenol are the major compoundsrecorded from the cladode extract of *Casuarinaequisetifolia,* whose result correlated with Salie*et al.* (1996). A study on phytochemical screening conducted by Moazzem Hossen *et al.* (2014) in methanolic cladode extract of *Casuarinaequisetifolia,* clearly marked the presence of flavonoids, alkaloids, steroids, saponin and tannin.

Conclusion and recommendations

Thus the brief study on secondary metabolite screening of the cladode extracts of *Casuarinaequisetifolia,* using polar solvent (aqueous, ethanol and methanol) reveals the presence of various pharmacologically effective phytochemicals.Ethanolic extracts proves to be a perfect warehouse for major secondary metabolites from this study. Thus this study could be extended to isolate pharmacologically applicable potential phytochemical, which could serve as precursor for synthetic medicine, to formulate a drug to cure wide range of human diseases.

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