



Preliminary phytochemical screening of alcoholic extract of stem bark of *Anamirta cocculus* Willd

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ABSTRACT

Phytochemical screening of plants or plant parts with reported medicinal and therapeutic properties could be highly beneficial in proposing their pharmacological actions. *Anamirta cocculus* Willd is a well known medicinal plant and belongs to family Menispermaceae. The purpose of the present study was to carry out phytochemical screening of alcoholic extract of stem bark of *Anamirta cocculus*. A crude alcoholic extract was obtained from the stem bark of *Anamirta cocculus* Willd., family Menispermaceae and was subjected to preliminary phytochemical screening. Qualitative tests were conducted for the detection of phytochemicals and the results showed that the alcoholic extract contains sugars and carbohydrates, flavonoids, alkaloids, saponins, steroids, tannins and phenolic compounds. Based on the results of phytochemical screening, appropriate pharmacological evaluation of the extract could be carried out for the identification and separation of potent bioactive phytoconstituents of *Anamirta cocculus*.

Keywords: *Anamirta cocculus*, Phytochemical screening, Stem bark, Ethanolic extract

INTRODUCTION

Data on Ayurveda and traditional could be logically employed in drug development. It would enable reduction of cost and save valuable time in drug development research. Chemical moieties from natural products could be used for development of new drugs. Under this scenario, phytochemical screening of plants or plant parts with reported medicinal and therapeutic properties could be highly beneficial. These types of screening are useful in proposing some pharmacological actions based on their chemical characteristics. *Anamirta cocculus* Willd is a southeast Asian and Indian climbing plant and is a well known medicinal plant. It belongs to family Menispermaceae. Therefore the aim of the present study was to carry out phytochemical screening of alcoholic extract of stem bark of *Anamirta cocculus*.

EXPERIMENTAL SECTION

Plant collection and taxonomical identification

The plant material for the proposed study '*Anamirta cocculus* Willd' was collected from the Herbal Garden of College of Pharmaceutical sciences, Medical College campus, Thiruvananthapuram, India, during the month of May 2006.

The species of the proposed study was identified as '*Anamirta cocculus* Willd' belonging to the family Menispermaceae by Regional Research Institute (Drug Research), Poojapura, Thiruvananthapuram, India.

Drying

The fresh stem barks were spread in trays and air dried for three weeks. The dried stem bark was powdered and sieved for extraction.

Method of Extraction

About 300 g of coarsely powdered stem bark of *Anamirta cocculus* was packed in a filter paper to form a thimble. The thimble was loaded in soxhlet extractor and defatted with petroleum ether (60-80°C), repeated three times. The petroleum ether defatted powder was packed in a soxhlet and extracted with 2.5 L of 90% ethanol at controlled temperature until completely exhausted. When all the powder (3 batches) was extracted, the extracts were combined and 80% of the solvent was recovered by distillation. The remaining solvent was removed by vacuum oven. This product was pasty in consistency and it was stored free from moisture in a desiccator over activated silica gel [1-3]. Physicochemical properties and yield of the extract were noted.

Phytochemical screening

The prepared alcoholic extract of stem bark of *Anamirta cocculus* was subjected to various chemical tests [4].

Tests for carbohydrates

Tests for carbohydrates were carried out as follows [5,6].

Molisch's test (General test)

To 2 mL aqueous dispersion of extract, added few drops of alpha naphthol solution in alcohol, shaken and added concentrated sulphuric acid through the side of the test tube.

Tests for reducing sugars

Benedict's test: Aqueous dispersion (5 mL) of alcoholic extract was treated with 5 mL Benedict's solution and heated in boiling water bath for two minute and cooled.

Fehling's test: Aqueous dispersion (2 mL) of extract was treated with 2 mL of Fehling's solution and heated on boiling water bath for 2 minutes, and then cooled.

Barfoed's test (Test for monosaccharide): Mixed equal volumes of Barfoed's reagent and test solution. Heated for 1-2 minutes in boiling water bath and cooled.

Tests for non-reducing polysaccharides (starch)

Iodine test: Mixed 3 mL aqueous dispersion of extract and a few drops of dilute iodine solution.

Test for Amino acids

To 2 mL of aqueous solution of alcoholic extract of *Anamirta cocculus* added 1 drop of Ninhydrin reagent (1% w/v).

Test for proteins

Biuret test (general Test): To 3 mL test solution, added 4% sodium hydroxide solution and few drops of 1 % copper sulphate solution.

Millon's test (for proteins): Mixed 3 mL test solution with 5 ml Millon's reagent.

Test for Glycosides

A small quantity of the extract was heated with dilute hydrochloric acid for 1 hour. To the hydrolysate, added 2 mL pyridine and 2 mL of sodium nitropruside solution. The mixture was made alkaline with sodium hydroxide solution [7].

Test for steroids

Salkowski reaction: To 2 mL of extract, added 2 mL chloroform and 2 mL concentrated sulphuric acid and shaken well.

Liebermann's reaction: Mixed 3 mL extract with 3 mL acetic anhydride. Heated and cooled. Added a few drops of concentrated sulphuric acid.

Test for flavonoids

Tests for flavonoids were carried out as follows [8].

(a) 1 drop of extract was coated on filter paper and showed in vapours of ammonia.

(b) **Lead acetate test:** To small quantity of residue added lead acetate solution.

(c) **Shinoda test:** To the alcoholic extract added 5 mL of 95% ethanol, few drops conc. HCl and 0.5 g magnesium turnings.

Test for tannins and phenolic compounds

Tests for tannins and phenolic compounds were carried out as follows [9].

(a) To 1 mL of gelatin solution, added 1ml of aqueous solution of *Anamirta cocculus* extract.

(b) To 2 mL of aqueous solution of alcoholic extract of *Anamirta cocculus* added 0.5 ml of ferric chloride solution.

Test for saponins

(a) *Foam test*: 1 mL of the extract was mixed with 20 mL of distilled water in a 100 mL measuring cylinder and shaken for 15 min.

(b) *Haemolysis test*: To 3 drops of blood added 1 drop of aqueous solution of extract and observed through microscope.

Test for Alkaloids

A little of the dry extract was dissolved in few drops of dilute HCl acid and filtered. The filtrate was tested for the presence of alkaloids [8,10].

(a) *Mayer's test*: Mixed 1 ml of the filtrate with 2-3 drops of Mayer's reagent in a watch glass.

(b) *Dragendroff's test*: To 2-3 drops of the filtrate on a white tile, added equal volume of Dragendroff's reagent and mixed.

(c) *Hager's test*: One ml of the filtrate was mixed with equal volume of Hager's reagent.

RESULTS AND DISCUSSION

Physicochemical properties and yield of the extract

An extract with thick and pasty consistency was obtained with blackish brown color and pungent odor. The yield of the alcoholic extract was found to be 2.88% w/w. The obtained extract was sparingly soluble in sodium hydroxide (10%), partially soluble in distilled water, partially soluble in hydrochloric acid (10%), and soluble in alcohol (20%). The p^H of extract (1%) in distilled water, 10% sodium hydroxide and 10% hydrochloric acid was determined to be 5.4, 12.06 and 0.73 respectively.

Phytochemical screening

Preliminary phytochemical screening of ethanolic extract of stem bark of *Anamirta cocculus* was carried out. The results of preliminary phytochemical screening are displayed in Table 1.

Tests for carbohydrates

Molisch's test (general test)

A Reddish violet ring at the junction indicated presence of carbohydrates in the extract.

Tests for reducing sugars

Benedict's test: A yellow/orange/red color indicated presence of reducing sugars in the extract.

Fehling's test: A red precipitate indicated the presence of reducing sugars in the extract.

Barfoed's test (Test for monosaccharide): No brick red precipitate indicating the absence of monosaccharides.

Tests for non-reducing polysaccharides (starch)

Iodine test: No blue/violet color was observed indicating the absence of starch.

Test for amino acids

No bluish violet color was developed on addition of Ninhydrin reagent indicating the absence of amino acids.

Test for proteins

Biuret test (General Test): No violet or purple color was observed indicating the absence of proteins in the extract.

Millon's test (for proteins): No red precipitate or solution was observed indicating the absence of proteins in the extract.

Test for glycosides

No pink to red color was observed when the mixture was made alkaline with sodium hydroxide solution indicating the absence of cardiac glycosides.

Test for steroids

Salkowski reaction: Chloroform layer appeared red and acid layers showed greenish yellow fluorescence indicating the presence of steroids in the extract.

Liebermann's reaction: Blue color appeared indicating the presence of steroids in the extract.

Test for flavonoids

(a) A yellow spot was observed when 1 drop of extract was coated on filter paper and showed in vapours of ammonia indicating the presence of flavonoids.

(b) *Lead acetate test*: A yellow colored precipitate was formed indicating the presence of flavonoids in the extract.

(c) *Shinoda test*: A pink color was observed indicating the presence of flavonoids in the extract.

Test for tannins and phenolic compounds

(c) A precipitate was formed when 1ml of aqueous dispersion of *Anamirta cocculus* extract was added to 1 mL of gelatin solution indicating the presence of tannins.

(d) On addition of ferric chloride solution a blue color was observed indicating presence of phenolic compounds.

Test for saponins

(a) *Foam test*: Formation of 1 cm of foam indicated the presence of saponins.

(b) *Haemolysis test*: Hemolysis occurred indicating the presence of saponins in the extract.

Test for Alkaloids

(a) *Mayer's test*: A pale yellow color indicated the presence of alkaloids in the extract.

(b) *Dragendroff's test*: An orange brown precipitate indicated the presence of alkaloids in the extract.

(c) *Hager's test*: A yellow precipitate indicated the presence of alkaloids in the extract.

Table 1: Chemical constituents present in crude alcoholic extract of *Anamirta cocculus*

Sl. No	Group of compounds tested for	Name of test/reagent(s) used	Presence (+) or Absence (-)
1	Sugars and Carbohydrates	Molish's test	+
		Benedict's test	+
		Fehling's test	+
		Barfoed's test	-
		Iodine test	-
2	Amino acids	Ninhydrin	-
3	Proteins	Biuret test	-
		Millon's test	-
4	Glycosides	Legal's	-
5	Steroids	Salkowski reaction	+
		Liebermann's	+
6	Flavonoids	Ammonia vapour	+
		Lead acetate	+
		Shinoda test	+
7	Tannis & Phenolic compounds	Gelatin	+
		Ferric chloride	+
8	Saponins	Foam test	+
		Haemolysis	+
9	Alkaloids	Mayer's test	+
		Dragendroff's test	+
		Hager's test	+

CONCLUSION

A crude alcoholic extract was obtained from the stem bark of *Anamirta cocculus* Willd., family Menispermaceae and was subjected to preliminary phytochemical screening. Qualitative tests were conducted for the detection of phytochemicals and the results showed that the alcoholic extract contains sugars and carbohydrates, flavonoids, alkaloids, saponins, steroids, tannins and phenolic compounds. Based on the results of phytochemical screening, appropriate pharmacological evaluation of the extract could be carried out for the identification and separation of potent bioactive phytoconstituents of *Anamirta cocculus*.

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