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In vitro antioxidant studies and phytochemical screening on the seeds of *Corchorus trilocularis*

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ABSTRACT

The current scientific investigation deals with the detection of class of compounds present in the seeds of corchorus trilocularis. The free radical scavenging activity was also determined using the DPPH assay method and the EC50 value were calculated and found to be 8.25mg .The seed of the plant is being used by the traditional practitioners and the present work reveals the scientific validation of the usage of the seeds.

INTRODUCTION

Herbal drugs referred as plants materials or herbals, involves the use of whole plants or parts of plants, to treat injuries or illnesses[1]. Herbal drugs are use of therapeutic herbs to prevent and treat diseases and ailments or to support health and healine[2]. These are drugs or preparations made from a plant or plants and used for any of such purposes. Herbal drugs are the oldest form of health care known to mankind[3]. There are many herbal products offered that assert to treat the symptoms of a broad range of problems, from depression to cold and flu. World Health Organization (WHO) has distinct herbal drugs as complete, labeled medicinal products that have vigorous ingredients, aerial or secretive parts of the plant or other plant material or combinations[4].

In India, Ayurveda medicine has used many herbs such as turmeric possibly as early as 1900 BC.Earliest Sanskrit writings such as the Rig Veda, and Atharva Veda are some of the earliest available documents detailing the medical knowledge that formed the basis of the Ayurveda system.Many other herbs and minerals used in Ayurveda were later described by ancient Indian herbalists such as Charaka and Sushtuta during the 1st millennium BC. The Sushruta Samhita attributed to Sushruta in the 6th century BC describes 700 medicinal plants, 64 preparations from mineral sources, and 57 preparations based on animal sources.[5]

Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods. Antioxidant activities are due to the presence of flavones, isoflavones, flavonoids, anthocyanin, catechins and isocatechins[6].

Antioxidants are phyto chemicals, vitamins and nutrients that protect our cell from damage caused by free radicals. They can be found in most fruits vegetables and medicinal herbs. Herbal drugs have gained importance in recent years because of the efficiency and cost effectiveness.

The plants are tall, usually annual herbs, reaching a height of 2–4 m, unbranched or with only a few side branches. The leaves are alternate, simple, lanceolate, 5–15 cm long, with an acuminate tip and a finely serrated or lobed margin. The flowers are small (2–3 cm diameter) and yellow, with five petals; the fruit is a many-seeded capsule. It thrives almost anywhere, and can be grown year-round[8].

The genus *Corchorus* is classified under the subfamily Grewioidea of the family Malvacea. It contains around 40 to 100 species[9].*Corchorus trilocularis* occurs in a wide range of habitats, from seasonally inundated land on clay soils and river banks to grassland, roadsides and disturbed places, and from black cotton soil to semi-arid sandy soils, as long as there is a warm season. It is most common in places with residual moisture such as clay plains, where it may colonize the entire area, becoming the most abundant species. It is commonly found as a weed in irrigated fields. It appreciates high temperatures as long as there is adequate moisture in the rooting zone.

MATERIALS AND METHODS

PREPARATION OF PLANT EXTRACTS AND PHYTOCHEMICAL SCREENING

The dried seed of Corchorus trilocularis subjected for air dried and make up to coarse powder form. Bark powder was extracted successively with methanol using Reflux apparatus. All the extracts were filtered using filter paper. The extracts were concentrated. The extracts were stored in air tight container. The seed extract of Corchorus trilocularis were analysed for the presence of sterols, alkaloids and amino acids.

PRELIMINARY PHYTOCHEMICAL SCREENING OF THE CORCHORUS TRILOCULARIS

The seeds of Corchorus trilocularis is taken 10g in 50ml methanol and subjected to extraction. The filtrate was subjected to Molisch's test. Formation of reddish brown ring indicated the presence of carbohydrates.

Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution [brown colour indicated the presence of carbohydrate.]

* Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours. Blue coloration of the spot indicated the presence of phenols.

Test for flavonoids: Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added .A pink or red coloration of the solution indicated the presence of flavonoids in the drug.

* Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added. Flocculent white precipitate indicated the presence of flavonoids.

Test for tannins

* Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the drug.

Test for steroid/terpenoid

* Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids.

Test for alkaloids

* Draggendorf's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Draggendorf's reagent. Orange coloration of the spot indicated the presence of alkaloids.

* Hager's test: The extract was treated with few ml of Hager's reagent. Yellow precipitation indicated the presence of alkaloids.

* Wagner's test: The extract was treated with few ml of Wagner's reagent. The reddish brown precipitation indicated the presence of alkaloids.

Tests for Glycosides

* Legal's test: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution. Pink to red colour solution indicates the presence of glycosides.

Test for Saponins

* Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes.A1cm layer of foam formation indicates the presence of Saponins

Test for Anthraquinones

* Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. Pink or red coloration of aqueous layer indicated the presence of Anthraquinones.

Test for Amino acids

* Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent. Blue colour indicated the presence of amino acids.

Test for fixed oils and fats

Press small quantity of the petroleum ether extract between two filter paper.Oilstains on the paper indicated the presence of fixed oils.

Note: the results for the above experiments can be noted as follows.

* If the response to the test is high it can be noted as +++which indicates that the particular group is present as the major class.
* If the response is average then note it as ++ indicates the presence in moderate quantity.
* If the response is very small then note it as + indicating the presence of only in traces.
* If no response is then negative.

IN-VITRO ANTIOXIDANT ASSAY

The percentage of antioxidant activity (AA %) of each substance was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams et al. The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UVVIS spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA). The mixture of ethanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA %) was determined.

RESULTS

CLASS OF COMPOUND	TESTS PERFORMED	RESULTS
Carbohydrates	Molisch's test Fehlings test	--- ---
Phenols	Phosphomolybdic acid test	+++
Flavanoids	Shinoda test lead acetate test	--- ---
Tannins	Braemer's test	---
Sterols	Salkowski's test	+++
Alkaloids	Draggendrof's test	++
Glycosides	Legals test	---
Saponins	Foam test	++
Anthraquinones	Borntragers test	---
Amino acid test	Ninhydrin test	
Fixed oils and fats		---

Table:1 Preliminary phytochemical screening of the seeds of C.trilocularis

Con in mg	Percentage Inhibition
0	0.00
5	41.25
10	49.50
15	57.75
20	66.00

Table:2 In-vitro anti-oxidant activity of *C.trilocularis*

CONCLUSION

We conclude that the seed of Corchorus trilocularis is a highly potential drug in terms of biological activity. The plant also contains promising class of compounds like alkaloids, phenols, flavones and sterols. The EC50 value which we calculated for the free radical scavenging was significant and we recommend further phytochemical studies on the seeds,which may lead to the identification of new potent molecules.

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