



PRELIMINARY PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF THE BARK OF INDIAN TRUMPET FLOWER

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ABSTRACT

The Indian trumpet flower *Oroxylum indicum* is a holistic plant used since long time as astringent, acrid, refrigerant, aphrodisiac, expectorant, carminative, digestive, anti-helminthic, diaphoretic, diuretic, anti-arthritis, anti-diabetic, and febrifuges. The extract of the plant is an important component of various folklore medicines used for treatment of stomatitis, nasopharyngeal cancer and tuberculosis. The phytochemical analysis of the bark of the plant was done to give a preliminary idea on the possible bioactive group of compounds present to validate the resourcefulness of the plant for treating a wide range of disease including cancer. The phytochemical analysis performed for the presence of various bioactive compounds revealed presence of glycosides, flavanoids, sterols, quinines, fats and oils and revealed very little presence of alkaloids. These groups of secondary metabolites are known to possess various medicinal qualities that prove the potentiality of the plant for curing wide range of diseases including cancer.

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INTRODUCTION

From the early inception, the knowledge of the traditionally used medicinal plants has been the source of many important drugs of modern time. Inclination of human beings towards Nature for various medicines is well known for preventing various diseases. World Health Organization (WHO) figures that up to 80% of the global population still depends on plants for primary health care. Almost 7000-7500 plants found native to the Indian Subcontinent are used as a constituent of medicinal preparations of various sections of people including the tribal communities (Mao *et al.*, 2009; Joy *et al.*, 1998). The North East region of India has more than 130 major tribal communities living in harmony with the environment (Dutta and Dutta, 2005). These tribal communities have scrutinized the rich flora and fauna around them throughout ages have developed valuable information on use of plants and plant products as herbal remedies for various ailments (Kala *et al.*, 2005). The exploration of this knowledge through scientific evaluation may contribute towards development of new novel drugs (Rates, 2001; Suba *et al.*, 2004).

Oroxylum indicum is one such holistic plant used since long time for the treatment of various diseases. In India the plant finds its huge application in various folklore medicines. Traditionally the decoction of the bark is given to the patients for the treatment of gastric ulcer.

The bark paste of the plant is used topically for getting relief from scabies and other skin diseases. The bark decoction is also taken for the treatment of mouth cancer. The seed is grounded with fire-soot and the paste is applied to neck for quick relief of tonsil pain (Joshi *et al.*, 2014). Chutney from mature fruits are prepared and is taken orally for the treatment of pharyngodya, cardiac disorders, gastropathy, bronchitis, haemorrhoids, cough, piles, jaundice, dyspepsia, small pox, leucoderma and cholera (Warnier *et al.*, 1995). Dried seed powder is used by women as purgative. The seeds grounded with fire soot to prepare a paste is used for curing tonsillitis. The seed oil along with sesame oil are instilled into ears mitigates the pain in otitis. (Chauhan, 1999). The decoction of the roots is used as astringent, acrid, refrigerant (Yoganarasimhan, 1996), aphrodisiac, expectorant, carminative, digestive, anti-helminthic, diaphoretic, diuretic, anti-arthritis, anti-diabetic, and febrifuges. Tonic from the whole plant is used in dropsy, cough, sprains, neuralgia, hiccough, colic, diarrhoea, strangury, gout, vomiting, leucoderma, wounds, rheumatoid arthritis and fever. The extract of root bark is used for treatment of stomatitis, nasopharyngeal cancer and tuberculosis (Bhattacharjee, 2005; Khare, 2004). The bark decoction is given to animals for deworming. The seed oil is used in perfume industry. It is to be noted that the bark extract of the plant is also used by the various tribal communities for the treatment of various cancer like symptoms.

The phytochemical analysis of the bark of the plant was done to have a preliminary idea on the possible bioactive group of

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compounds present to validate the resourcefulness of the plant for treating of various diseases including cancer.

METHOD AND METHODOLOGY

Preparation of bark extract of *Oroxylum indicum*

The barks of healthy plant *Oroxylum indicum* were collected from Krishnanagar locality, Guwahati, Assam during Nov, 2013. These barks were washed with clean distilled water for removing the adhering dust particles. They were then chopped into small pieces and were dried in the shaded place. The dried barks were powdered in a grinder. For extraction by the method of maceration, 5 gm of bark powder was taken in a beaker and 50 ml of 70% ethanol was added to it as the extraction solvent and allowed to macerate in the dark for 72 hrs at room temperature using a magnetic stirrer at 1200 rpm. Filtration was done and the filtrate was collected in a conical flask. Ethanol was again added to the residue and stirred. The process was repeated for 4-5 times. The total filtrate was transferred to a round bottom conical flask and then dried in a heating mantle but not evaporated to absolute dryness (Trusheya *et al.*, 2007). The concentrated extract was taken in a falcon tube and then lyophilized to powder form in a period of 36 hrs. 15ml of the extract produced an yield of 0.45g.

Phytochemical analysis

The phytochemical analysis of the bark was carried out to determine the presence of following bioactive compounds using the standard qualitative procedures (Trease and Evans, 1989; Harbone 1998).

Tests for Alkaloids

Dragendorff's test: To 1 ml of each of the sample solution taken in a test tube few drops of Dragendorff's reagent (potassium bismuth iodide solution) was added. Presence of alkaloids is indicated by the formation of a reddish brown precipitate.

Hager's test: To 1 ml of each of the sample few drops of Hager's reagent (Picric acid) was added, formation of yellow precipitate indicated presence of alkaloids.

Tests for glycosides

Bromine water test: The test solution was treated with bromine water. Presence of glycosides gives a yellow precipitate.

Kellar Kiliani test: 1ml of concentrated sulphuric acid was taken in a test tube and then added to it 5ml of extract and 2ml of glacial acetic acid with one drop of ferric chloride. Formation of a blue colour indicates glycosides.

Tests for Flavanoids

Lead acetate test: Aqueous basic lead acetate was added to test sample. It produces reddish brown precipitate if flavanoids are present.

Ferric chloride test: To few ml of test samples taken separately, few drops of ferric chloride were added. It forms a blackish red precipitate if flavanoids are present.

Tests for sterols: Salkowski test: Few drops of concentrated sulphuric acid were added to the test samples in chloroform, a red colour appears at the lower layer indicates the presence of sterols.

Tests for Quinones

When alcoholic KOH was added to the test samples. The appearance of red to blue colours indicates the test solution reacting positively for quinines.

Tests for fats and Oils

Stain test: A small quantity of each extract between two filter papers, the stain on filter papers indicates the presence of the oils.

RESULTS

The results of the phytochemical screening of the bark extract of *O. indicum* for testing the presence of different secondary metabolites are given in Table 1.

Table 1 Analysis of phytochemicals of bark extract of *O. indicum*

Phytochemicals	Tests	Observations	Inference
Alkaloids	Dragendorff's test	-	Absence of alkaloids
	Hager's test	-	
Glycosides	Bromine water test	+	Presence of glycosides
	Kellar Kiliani test	+	
Flavanoids	Lead acetate test	+	Flavanoids may be present
	Ferric chloride test	-	
Sterols	Salkowski test	+	Presence of flavanoids
Quinones	Alcoholic KOH test	+	Presence of quinones
Fats and oils	Blotting paper technique	+	Presences of oils and fats

The phytochemical analysis of the bark extract of *O. indicum* revealed the presence of glycosides, flavanoids, sterols quinines, fats and oils and revealed very little presence of alkaloids.

DISCUSSION

The secondary metabolites in the plant kingdom such as polyphenols, flavanoids and brassinosteroids have been extensively investigated for their potentiality to combat various diseases. Collectively they have been known to show wide range of properties such as anticancer properties which include antioxidant activity; inhibition of cancer cell growth; induction of apoptosis; target specificity; cancer cell cytotoxicity (Gupta *et al.*, 2014; Cao *et al.*, 2013; Malíková *et al.*, 2008; Kumar *et al.*, 2014). Many plant-derived drugs have been developed from positive results in research and have progressed into clinical trials. Drugs obtained from vinca alkaloids were among the first compounds to be utilized and are developing in clinical Phase III trials along with paclitaxel and other anticancer agents. These compounds are easily available from the natural environment and are relatively non-toxic to healthy human cells (Greenwell and Rahman, 2015).

During the qualitative phytochemical estimation of the bark extract of OI, we have found that the bark extract tested positive for the presence of important phytochemicals like glycosides, flavanoids, sterols, quinines along with presence of small amount of fats and oils. This result is at par with the investigation carried out by Ramaswamy *et al.*, 2014 which showed the presence of flavonoids, alkaloids, glycosides, tannins, sterols, phenols, saponins, fats and oils in fresh and dry leaf extracts of *O. indicum* (Ramaswamy *et al.*, 2014). The presence of flavonoids and glycosides indicates the possibility of OI to have therapeutic properties. Purified flavonoids exhibit anticancer activities against human cancers including

hepatoma (Hep-G2), cervical carcinoma (Hela) and breast cancer (MCF-7) (Wen *et al.*, 2014).

Brassinosteroids (BRs), the precursors of plant steroids (Clouse, S. D., 2011) are naturally occurring compounds found in plants which play roles in hormone signalling to regulate growth and differentiation of cells, elongation of stem and root cells and other roles such as resistance and tolerance against disease and stress (Greenwell and Rahman, 2015). BRs have been used in investigations to treat a range of cancer cell lines which include T-lymphoblastic leukaemia CEM, multiple myeloma RPMI 8226, cervical carcinoma HeLa, lung carcinoma A-549 and osteosarcoma HOS cell lines. BRs were also found to be effective in the treatment of cell lines in breast cancer and prostate cancer (Malíková *et al.*, 2008). OI extract contains quinones which are known to have various anti-proliferation and anti-metastasis effects in various cancer types both *in vitro* and *in vivo* (Lu *et al.*, 2013). Thus, the presence of various active ingredients (secondary plant metabolites) as revealed by the phytochemical screening (table 1) supports the resourcefulness of *Oroxylum indicum* against various diseases including cancer.

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