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Comparative *in-vitro* anti arthritic studies on the various extracts of *Glycosmis pentaphylla* dc roots

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ABSTRACT

Glycosmis pentaphylla DC is a medicinal plant widely used in traditional systems of medicine for the treatment of arthritis. The present study deals with the investigation on the roots of *Glycosmis pentaphylla* DC (Rutaceae) for its anti-arthritic potential. To evaluate the anti-arthritic efficacy, the chloroform, ethyl acetate and ethanol extracts of roots of *Glycosmis pentaphylla* were taken and screened by bovin serum albumin denaturation method. A preliminary phytochemical screening revealed the presence of active phytochemicals such as alkaloid, glycosides, terpenoids, flavonoids tannins and phenolic compounds in the different extracts. The results indicated that all the extracts tested, have shown positive response, with the ethanol extract exhibiting maximum efficacy of 46.46%, when compared with standard drug, diclofenac sodium with 51.92 % inhibition at 10 µg/ml. The effect of different extracts were in the order of ethanol > ethyl acetate > chloroform. The study concludes that further investigational studies are required to identify the phytoconstituent responsible for the activity and to elucidate the exact mechanism of anti-arthritic activity in *Glycosmis pentaphylla*.

Keywords: *Glycosmis pentaphylla*, anti-arthritic activity, ethanolic extract, protein denaturation method

INTRODUCTION

Arthritis is a painful swelling of joints and it is a common disease affecting large population. Osteoarthritis, rheumatoid arthritis and gout are some of the common types prevalent worldwide, each occurring at different stages and involved with different mechanism of action. The revival of herbal and other complementary therapies in the management of chronic diseases such as RA and other inflammatory disorders is well documented[1]. However, despite an increase in use, evidence for the effectiveness and safety of these complementary therapies is limited. A number of plant species still remain to be surveyed systematically for their biologically active chemical compounds. A thorough phytochemical analysis is needed to isolate useful ingredients from traditional herbal drugs. Although there are numerous reports on plant based ailments and cure, still there is a continuous need to identify newer leads from traditional sources with significant therapeutic efficacy.

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Glycosmis pentaphylla is a flowering plant in the citrus family (Rutaceae) known commonly as orange berry and gin berry. Juice of its leaves is used in fever, liver complaints and as a vermifuge, while leaves are considered good antidote for eczema and other skin troubles[2]. *Glycosmis pentaphylla* has also been found to have antioxidant[3, 4, 5, 6], galactagogue, immune stimulant[7], larvicidal[8], antipyretic[9, 10] and hepatoprotective properties[11, 12, 13, 14, 15]. In folk medicine, the bark of *Glycosmis pentaphylla* is used for the treatment of diabetes and gonorrhea. It is well evident that the roots are useful in the treatment of arthritis[16, 17].

Hence, we attempted to evaluate and compare the anti-arthritic potential of the different extracts of the roots of *Glycosmis pentaphylla*, as a prelude for further studies.

MATERIALS AND METHODS

Collection of material

The plant was collected from Thirumala thirupathi forests of Western Ghats, Chittoor District, Andhra Pradesh during the month of September 2010 and authenticated by Prof P.Jayaraman, Plant Anatomy Research Centre, West Tambaram, Chennai. A voucher specimen has been kept in Department of Pharmacognosy, Faculty of Pharmacy, Sri Ramachandra University for future reference. The root of the plant

were separated, dried at room temperature and subjected to communition to get a coarse powder.

Preparation of extract

The coarsely powdered root material was subjected to successive solvent extraction[18] for 72 hours using various solvents of increasing polarity such as chloroform (1), ethyl acetate (2) and ethanol (3) by cold maceration process. The above solvents (1-3) was concentrated by rotary vacuum evaporator and stored in desiccators until use.

Phytochemical analysis

A preliminary qualitative phytochemical screening was performed[19] for the three extracts (1-3) for identifying the presence of phytochemicals such as alkaloids, flavonoids, steroids, phenols, tannins, saponins (foam test), glycosides, resins, carbohydrates, proteins and amino acids.

***In vitro* anti-arthritic activity by inhibition of protein denaturation method**

The protein denaturation screening used in this study was adopted from the method of Singh M. et al. 2011[20]. A short summary of the method is briefed out.

Preparation of the test and standard solution:

The standard solutions (0.5 ml) were prepared using 0.45 ml of Bovine serum albumin (5 % w/v aqueous solution) and 0.05 ml 0f Diclofenac sodium.

Preparation of the test solution:

The test solutions (0.5 ml) were prepared using 0.45 ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of test solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml).

Preparation of the test control solution:

This solution (0.5 ml) was prepared using of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water.

Preparation of the test control solution:

This solution (0.5 ml) was prepared using of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of test solution.

Experimental

All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37 °C for 20 min and the temperature was increased to keep the samples at 57 °C for 3 min. After cooling, 2.5 ml of phosphate buffer was added to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm. The percentage inhibition of protein denaturation was calculated using the formula:

Percentage inhibition = [100-(optical density of test solution – optical density of product control) ÷ (optical density of test control)] ×100.

The control represents 100% protein denaturation. The results were compared with reference standard, diclofenac sodium (250 µg/ml). The percentage inhibition of protein denaturation of different extracts is tabulated in Table. 1.

RESULTS AND DISCUSSION

The phytochemical screening of various extracts of the roots of *Glycosmis pentaphylla* has shown the presence of alkaloids, phenols, flavanoids, glycosides and terpenoids and carbohydrate as shown in Table 1.

Table. 1. Preliminary qualitative phytochemical screening of various extracts of *Glycosmis pentaphylla* DC roots

S. No	Phytochemicals	Chloroform	Ethyl acetate	Ethanol
1.	Alkaloids	+	+	+
2.	Carbohydrate	-	+	+
3.	Glycosides	-	+	+
4.	Protein	-	-	-
5.	Amino acids	-	-	-
6.	Steroids	-	-	-
7.	Phenols	+	+	+
8.	Flavonoids	-	+	+
9.	Tannins	-	+	+
10.	Saponins	-	-	-
11.	Terpenoids	-	+	+
12.	Resins	-	-	-

The presence of phytoconstituents is represented as "+" and the absence of phytoconstituents is indicated as "-"

These extracts when subjected to *in vitro* anti-arthritic activity by protein denaturation method indicated that, the ethanolic extract has shown maximum inhibition, when compared to the reference standard diclofenac sodium. The significant activity exhibited by the ethanol fraction is explained due to the presence of flavanoids, phenols and tannins in it. The percentage protection of the various extracts (1-3) at 1000 µg/ml concentration was found to be 79.12± 3.2% (Chloroform), 82.93±3.0% (Ethyl acetate), 87.42±2.2% (Ethanol) and 94.02±2.1% (Diclofenac sodium). All the extracts have shown dose dependant response as tabulated in Table 2. All the extracts have shown dose dependant response as shown in Figure 1. The effect was represented in the order of Ethanol > Chloroform > Ethyl acetate.

Table. 2. *In vitro* anti arthritic activity in *Glycosmis pentaphylla* DC roots

Concentration (µg/ml)	% Inhibition			
	Chloroform	Ethyl acetate	Ethanol	Diclofenac Sodium
10	38.42±1.8	40.20±2.0	43.46±3.1	51.92±0.4
50	49.28±1.9	51.17±2.5	50.53±4.0	57.68±0.5
100	55.02±2.1	60.74±2.1	60.35±2.5	63.03±0.9
200	67.67±2.6	67.07±0.9	67.39±3.0	79.52±3.5
400	73.29± 3.0	74.84±3.5	73.32±2.4	85.02±0.8
800	72.12±2.6	78.37±2.5	81.13±3.3	92.87±1.5
1000	79.12± 3.2	82.93±3.0	87.42±2.2	94.02±2.1

Values are expresses as Mean ± Standard Deviation in triplicates

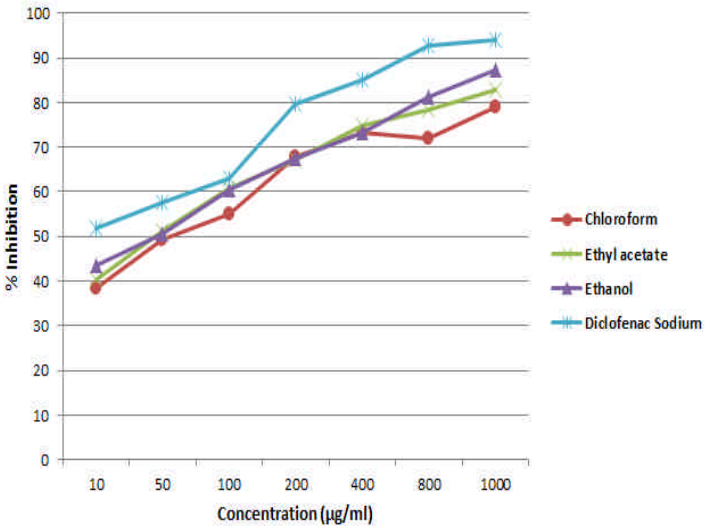

Fig. 1. *In vitro* anti arthritic activity in *Glycosmis pentaphylla* DC Roots

The Figure 1 represents the dose dependant response curves of the various extracts of Glycosmis pentaphylla DC Roots against the reference standard Diclofenac sodium. Denaturation of protein is one of the causes of rheumatoid arthritis and is well documented. Production of auto antigen in certain arthritic disease may due to denaturation of protein. The mechanism of denaturation probably involves alteration of electrostatic hydrogen, hydrophobic and disulphide bonding. From the results of the present study, it can be stated that all the extracts of *Glycosmis pentaphylla* roots are capable of controlling the production of auto antigen involved in the inhibition of the denaturation of proteins. The study also confirms the traditional claim of *Glycosmis pentaphylla* as an anti-arthritic drug. There are several reports suggesting the wide biological activities of the secondary metabolites of *Glycosmis pentaphylla*[21-30]. Further studies need to be done for the identification of active principles responsible for the anti-arthritic potential and *in vivo* evaluation need to be performed for enumerating the exact mechanism of action contributing to the arthritic potential of the roots of the plant, *Glycosmis pentaphylla*.

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