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PHARMACOGNOSTICAL, PHYTOCHEMICAL EVALUATION AND ANTIARTHRITIC ACTIVITY OF *GLYCOSMIS ARBOREA*

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ABSTRACT

*Glycosmisarborea*DC is a medicinal plant widely used in traditional systems of medicine for the treatment of arthritis. To evaluate the anti-arthritic efficacy, the chloroform, ethyl acetate and ethanol extracts of roots of *Glycosmisarborea* were taken and screened by bovin serum albumin denaturation method. The results indicated that all the extracts tested, have shown positive response, with the ethanol extract exhibiting maximum efficacy of 48.46%,when compared with standard drug, diclofenac sodium with 55.92 % inhibition at 10 µg/ml. The effect of different extracts were in the order of ethanol > ethyl acetate > chloroform.

Key Words: *Glycosmisarborea*, anti-arthritic efficacy

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INTRODUCTION

Little work was carried out by the pharmaceutical industry during 1950- 1980's; however, during the 1980-1990's massive growth has occurred. This has resulted in new developments in the area of combinational chemistry, new advances in the analysis and assaying of potential plant materials as drug leads by conservationists. New plant drug development programs are traditionally undertaken by either random screening or an ethno botanical approach, a method based on the historical medicinal/ food use of the plant. One reason why there has been resurgence in this area is that conservationists especially in USA have argued that by finding new drug leads from the rainforest, the value of the rainforests to society is proven and that this would prevent these areas being cut down for unsustainable timber use. However, tropical forests have produced only 47 major pharmaceutical drugs of worldwide importance. It is estimated that a lot more say about 300 potential drugs major importance may need to be discovered. These new drugs would be worth $ 147 billion. It is thought that 125000 flowering plant species are of pharmacological relevance in the tropical forests. It takes 50000 to one million screening tests to discover ONE profitable drug. Even in developed countries there is a huge potential for the development of nutraceuticals and pharmaceuticals from herbal materials. For example the UK herbal material medica contains around 300 species, where as the Chinese herbal material medica contains around 7000 species, one can imagine what lies in store in the flora- rich India (1-3).Arthritis is the swelling and tenderness of one or more of your joints. The main symptoms of arthritis are joint pain and stiffness, which typically worsen with age. The most common types of arthritis are osteoarthritis and rheumatoid

arthritis. Osteoarthritis causes cartilage — the hard, slippery tissue that covers the ends of bones where they form a joint — to break down. Rheumatoid arthritis is a disease in which the immune system attacks the joints, beginning with the lining of joints. Uric acid crystals, which form when there's too much uric acid in your blood, can cause gout. Infections or underlying disease, such as psoriasis or lupus, can cause other types of arthritis (4,5).The ethnobotanical information of the plant *Glycosmisarborea*has been existing for a quite a long time. The literature survey on *Glycosmisarborea*indicates that so far no detailed Pharmacognostic, anti-rheumatic, and antioxidant studies were carried out from the roots. Hence we aimed to carry out research on the roots of *Glycosmisarborea.*

MATERIALS AND METHODS

Collection of Plant Material

The roots of *Glycosmisarborea* was collected in the month of January from Tirumala hills, Tirupathi, Andhra Pradesh, Western ghats of South India.The required samples of different organs were cut and removed from the plant and fixed in FAA (Farmalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol – 90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of Paraffin wax (Melting point 58-60^{0}C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Preparation of Powder and Extract

The root was shade dried and pulverized in a mechanical grinder. The powder (1.5 kg) was successively extracted with various solvents such as petroleum ether (40-60^{0}C), chloroform, ethyl acetate, ethanol, 50 % hydro alcohol. The extracts were concentrated under reduced pressure in a rotary evaporator (Technico, India).

In vitro anti-arthritic activity by inhibition of protein denaturation method (6, 7)

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.

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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).

RESULTS AND DISCUSSION

In Vitro Anti-Arthritic Activityby Inhibition of Protein Denaturation Method

In arthritis auto antigens are produced due to proteinase action, denaturation of protein.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-1. 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-1*In vitro* anti arthritic activity in *Glycosmisarborea*DC roots

Concentration (µg/ml)	% Inhibition			
	Chloroform	Ethyl acetate	Ethanol	Diclofenac Sodium
10	39.42±1.8	41.20±2.0	48.46±3.1	55.92±0.4
50	49.28±1.9	51.17±2.5	52.53±4.0	59.68±0.5
100	57.02±2.1	62.74±2.1	62.35±2.5	65.03±0.9
200	67.67±2.6	67.07±0.9	67.39±3.0	79.52±3.5
400	75.29± 3.0	76.84±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	79.92± 3.2	82.93±3.0	87.42±2.2	94.02±2.1

Values are expresses as Mean ± Standard Deviation in triplicates

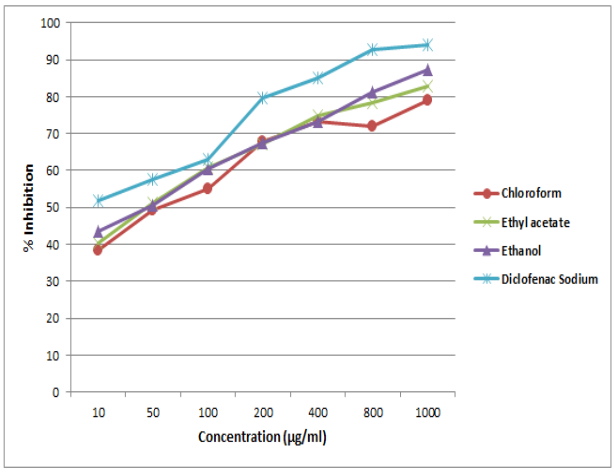

Fig-1*In vitro* anti arthritic activity in *Glycosmisarborea*

The graph represents the dose dependant response curves of the various extracts of *Glycosmisarborea* 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 *Glycosmisarborea*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 *Glycosmisarborea*as an anti-arthritic drug. There are several reports suggesting the wide biological activities of the secondary metabolites of *Glycosmisarborea*.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, *Glycosmisarborea*.

CONCLUSION

*Glycosmisarborea*DC is a medicinal plant widely used in traditional systems of medicine for the treatment of arthritis. A preliminary phytochemical screening revealed the presence of active phytochemicals such as alkaloid, glycosides, terpenoids, flavonoids tannins and phenolic compounds in the different extracts. To evaluate the anti-arthritic efficacy, the chloroform, ethyl acetate and ethanol extracts of roots of *Glycosmisarborea* were taken and screened by bovin

serum albumin denaturation method. The results indicated that all the extracts tested, have shown positive response, with the ethanol extract exhibiting maximum efficacy of 48.46%,when compared with standard drug, diclofenac sodium with 55.92 % 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 *Glycosmisarborea*.

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