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Phytochemical Characterization of Herbal Drug Formulation for Arthritis

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ABSTRACT

Siddha and Ayurveda are the two major Traditional Indian systems of Medicine. Siddha is widely practiced in the Tamil speaking areas of South India. The frequently prescribed Siddha medicine (Arthoguard) of herbal origin that developed against arthritis was selected and subjected to preliminary phytochemical screening. The extract of the drug was obtained by using ethanol in a maximum yield of phenol. Spectrophotometric analysis indicated that phenols ($2.033 \pm 0.05 \text{ mg mL}^{-1}$) are the prevalent compounds present in this herbal drug formulation. This result was confirmed with thin layer chromatography. To identify the exact type of phenolic constituents, mass spectrometry was carried out and the compounds were identified as dodecylacetate, hexadecanoic acid, farnesol and geranylacetate, of which geranylacetate was the principal constituent. This is the first such attempt to provide scientific documentation of this herbal drug. Further studies on the mode of action of these drugs, their immunomodulatory effect, studies related to the understanding the molecular basis of these mechanisms will lead us a long way to position herbal drugs in global market.

Key words: Herbal drug, arthritis, phytochemical, natural products

INTRODUCTION

Among the traditional Indian systems of medicine, Siddha is widely practiced in the Tamil speaking areas of South India. Siddha which is largely therapeutic in nature has a rich heritage and history. Ayurveda is an ancient medical science for the restoration of health and prevention of diseases (Patwardhan *et al.*, 2009; Sharma *et al.*, 2007). Many traditional medicines in use are derived from medicinal plants, minerals and organic matter (Joseph and Jini, 2011a). According to the World Health Organization (De Silva, 1997), about 80% of the population in many third world countries still uses traditional medicine (e.g., medicinal plants) for their primary health care, due to poverty and lack of access to modern medicine. Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drug (Benkeblia, 2004).

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind (Balasundaram *et al.*, 2011; Nebedum *et al.*, 2009; Joseph and Jini, 2011b). The root of the plant *Inula racemosa* has been used as folk medicine in East Asia and Europe to treat bacterial infections (Lokhande *et al.*, 2007). Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Zakaria, 1991; Sood *et al.*, 2009). To promote the proper use and to determine their potential as sources for new

drugs, it is essential to study the medicinal plants and their derivatives. Therefore, researchers are increasingly turning their attention to folk medicine to develop better drugs against many diseases (Parekh and Chanda, 2007).

There is a very clear note in the ancient Siddha literatures that the patient should be first treated with herbal products only (Kudineer, Chooranam, pills and etc.) and if the desired effect is not attained, then only metals, minerals and animal products should be used. In accordance to this, the frequently prescribed Siddha medicines of herbal origin was selected and subjected to preliminary phytochemical screening. Many herbal products, several metals and minerals have been used in an attempt to cure or control Arthritis, since ages. In recent years, there has been an upsurge of interest in herbs, spices and vegetables that contain natural substances with health-promoting or pharmaceutical properties. Many vegetables are sources of dietary antioxidants (Suhaj, 2006; Mian and Mohamed, 2001; Velioglu *et al.*, 1998) that may help to mitigate a range of chronic health problems by reacting with free radicals (Premeanth *et al.*, 2011). Ayurveda, Unani Chinese and other traditional medical systems, provide substantial lead to find active and therapeutically useful antioxidant compounds from plants (Sati *et al.*, 2010).

Arthroguard is a new anti-inflammatory combination drug prepared from plants. This unique formula of Arthroguard is specifically designed to replace the important lubricating and connective components of joint tissue. The present attempt is a scientific approach to analyse the phytochemical composition of this herbal drug formulated against arthritis. The potential outcome of this research is the identification of the bioactive compound from this herbal drug formulation that act against arthritis. This study enables the identification of potential phyto-chemicals involved in a combination of activities like anti-inflammatory activity, pain relieving effect with anti-oxidant property and with immuno modulating ability.

MATERIALS AND METHODS

Preparation of the extract: The selected drug Arthroguard is a new anti-inflammatory combination drug prepared by Prebhat Herbal Research Centre was used for the entire study.

Around 5 g of the sample was ground with a pestle and mortar, in 10 times volume of ethanol. Centrifuge the homogenate at 10,000 rpm for 20 min. Save the supernatant and the extraction was repeated for five times with the volume of 80% ethanol. The supernatants were pooled and extracted by centrifugation.

Quantitative analysis of phenol: Total phenol concentrations were determined using a Folin-Ciocalteu assay, as described by Amin *et al.* (2006). A 0.1 mL aliquot of sample (5 mg mL⁻¹ in methanol) was added to 0.75 mL of Folin-Ciocalteu reagent, which was previously diluted 10-fold with distilled water. The mixture was allowed to stand at room temperature for 5 min and then 0.75 mL of 10% sodium bicarbonate was added, followed by 10 mL of distilled water. After standing for 90 min at room temperature, absorbance was then measured at 725 nm. Gallic acid was used as the standard phenol and the total phenol concentration was expressed as gallic acid equivalents/mg of extract.

TLC screening: The extract was run on pre-coated silica gel plate using the mixture of acetic acid and chloroform in different proportion as the mobile phase and vanillin-H₂SO₄ reagent was used as spray reagent. R_f values were also calculated for every spots (Harbone, 1984).

Gas chromatography mass spectrometry (GCMS) analysis: Analysis by GC-MS was carried out in shimadzu GC 17A QP5000 by using DBS Gas chromatography glass column with electron multiplier detector with Quadrapole analyzer under the following run conditions. Flow rates zero, temperatures 70°C for 1 min followed by a flow rate of at 250°C for 10 min. Helium is used as the carrier gas at rate of 0.6 mL min⁻¹.

RESULTS AND DISCUSSION

The theme of this work is 'Scientific validation of drugs'. A drug is a chemical substance which is synthetic or extracted from a plant or animal used as a medicine to cure a disease. Plants provide us nearly 35% of the drugs, so that many pharmaceutical companies have collecting centers specifically for plants used as drugs in many forests of the third world and developing countries. The present work is a scientific approach to analyse the phytochemical composition of this herbal drug developed against arthritis. This study enables the identification of potential phyto-chemicals involved in a combination of activities like anti-inflammatory activity, pain relieving effect with anti-oxidant property and with immuno modulating ability.

The total phenol content in the herbal drug extract was estimated and it was found to be 2.033±0.05 mg mL⁻¹. The amount of phenol in plant samples is not a fixed value but fluctuates rapidly with changes in the stage of growth of plant, environmental conditions and stress conditions to which the plant is subjected. Gomathi *et al.* (2005) have reported similar fluctuations in phenolic content in jasmine plant. The level of phenol in relation to variation in incident light is a finely tuned process, which must be explained in terms of plant physiology and intermediate metabolism rather than in terms of resource allocation or a direct response to herbivory (Mole *et al.*, 1988). The concentration of phenol was higher in sorediate than in non-sorediate lobe ends of the lichens (Hyvarinen *et al.*, 2000). The leaves had higher phenol contents and the highest content reached at noon in all species of the genus *Hypericum* (Ayan *et al.*, 2007). The ontogenetic fluctuations in phenolic content were also reported for other plant species such as *Malus domestica* (Treuter, 2001), *Morus alba* and *Morus nigra* (Sivaci and Sokmen, 2004). Phenolic compounds are important for their contribution to the colour, sensory attributes and nutritional and antioxidant properties of plants (Chrestel *et al.*, 2000). Phenolic compounds are reported to have multiple biological effects, including antioxidant activity, antitumor, antimutagenic and antibacterial properties (Shui and Leong, 2002; Sathishkumar *et al.*, 2009).

The phytochemicals present in the plants are studied with the help of Thin Layer Chromatography (Satnami and Yadava, 2011; Badole and Bodhankar, 2009). In our study, we carried out the thin layer chromatography with pyro-catechol and phenol as standards and the herbal drug as test sample. Table 1 indicated that the test sample is a phenolic compound of different nature than the used standards. Therefore further analysis to identify the exact compounds present in the herbal samples can be found out only if other suitable standards are available.

Table 1: TLC profile of the herbal extract

No. of spots	Colour of the spot	Rf value
1	Black	0.714
2	Orange	0.501
3	Yellow	0.357
4	Violet	0.642

Table 2: Constituents of the extract from herbal drug, Arthroguard

Peak No.	Retention time	Compound	Peak area (%)
1	6.732	Dodecenylacetate	4.16
2	7.520	Hexadecanoic acid	6.47
3	11.251	Farnesol	22.95
4	12.851	Geranylacetate	66.42

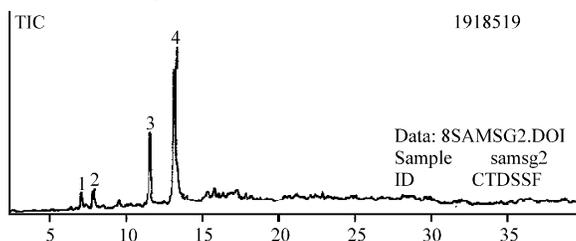


Fig. 1: GC-MS chromatogram of herbal drug, Arthroguard extract

The phytochemicals present in the herbal extracts were confirmed with the help of gas chromatography (Okiei *et al.*, 2009). In our study, the gas chromatography mass spectrometry analysis was carried out with different phenolic standards available with SPIC science foundation. The GC-MS chromatogram (Fig. 1) of the herbal extract showed four major peaks indicating the presence of four phytochemical constituents. On comparison of the mass spectra of the constituents with the available standards in the SPIC science foundation, the four phytoconstituents were characterized and identified. Table 2 indicated the presence of about four major compounds viz., dodecenylacetate, hexadecanoic acid, farnesol and geranylacetate with the retention time of about 12.821, 11.251, 6.73 and 7.52 min, respectively.

In order to promote Indian herbal drugs, there is an urgent need to evaluate the therapeutic potentials of the drugs as per WHO (2000) guidelines. Patwardhan *et al.* (2004) mentioned that 30% of drugs selling in the world's market are based on natural products. Traditional indigenous medicine is limited to small tribal and geographical areas called 'little traditions' are an excellent repository of knowledge about medicinal properties of botanical sources. Kamboj (2000) stated that the bioactive extract should be standardized on the basis of phytochemical compounds. Phytochemical screening of herbal drugs is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate urgent steps for screening of herbal drugs for the bioactive compounds. In the present communication, an attempt has been made to assess the status of phytochemical properties in the herbal drug (Arthroguard) against arthritis, which will be beneficial in standardization of drug discovery and development. This drug is used by the people extensively to improve the health status and also use in pharmaceutical and nutraceutical products of commercial importance.

CONCLUSION

This study provides a preliminary analysis on phytochemical characterization of the herbal drug for arthritis. This study need to be continued further to make further progress by fractions, chromatography and mass spectrometry analysis of purified fractions. Among the four compounds

present in the herbal extract whether all four independently contribute to join pain cure or a combination of these is yet to be found. This analysis of interest may be useful to both consumers and food scientists, as the herbal drug may provide health benefits to the public and also act as medicine for arthritis.

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