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Research Article

Evaluation of Antioxidant and Anti-inflammatory Potentials of Selected Siddha Herbal Drugs – An *In vitro* Study

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ABSTRACT

Herbal drugs such as cardamom (*Elletaria cardamomum* L.), ginger (*Zingiber officinale* Roscoe), arrow root (*Maranta arundinacea* L.), yew leaves (*Abies webbiana* (D. Don) Spach), Indian rose chestnut (*Mesua ferrea* L.), pepper (*Piper nigrum* L) and clove (*Syzygium aromaticum* (L.) Merrill & Perry) have been used in the preparation of various Siddha formulations. The afore-said herbal drugs exhibited therapeutic effect against the loss of appetite, indigestion, gastric reflex, hiccup, flatulence, itching, scabies, cold, cough, head-ache and joint pains. Even though these herbal drugs have been used to manage arthritis, there is no scientific evidence regarding mechanism of action. Hence, the present study dealt with the evaluation of antioxidant and anti-inflammatory potentials of selected herbal drugs through *in vitro* studies so as to provide scientific evidences for their anti-arthritic effect.

Key words: Herbal drugs, phytochemistry, polyphenols, antioxidant, anti-inflammatory.

INTRODUCTION

Arthritis is a joint disorder accompanied with pain, joint stiffness, inflammation, swelling and frequent changes in body structure due to the age, trauma or infection in the joints. According to Siddha system arthritis is called as Santhu vaatham, Kizh vayu, Moottu vali, Aamavatham, Megasulai and Mudakkuvayu[1]. Vatham is the causative factor for pain, which is classified into 85 types and the joint pain is called Santhu vaatham[2]. The affected person shows symptoms of joint pain, disable to walk, morning stiffness, difficult to move, muscle weakness, increase the pain after flex the affected joint and tenderness. Siddha literature describes that this disease has the symptoms like join swelling, pricking pain, disable to flex and fold the joint, body tiredness, giddiness, tongue dryness, or excessive salivary secretion[1]. Causes of arthritis are calcium deficiency, excessive usage of joints, aging, dryness of synovial fluid, fracture, oxidative stress and inflammation[3]. Vatham-induced arthritis was stimulated by excessive intake food substances like potato, banana, cold foods, venereal diseases, genetic disorders, exposure to chill weather and moist conditions [1].

The currently used drugs in Allopathic system are Diclofenac sodium and Ibrufen, but they have various side effects. In Siddha system *Amukkara chooranam, Thirikaduku chooranam, Panchadeepakini chooranam, Astathi chooranam Elathi choornam* and *Thiribala chooranam* are used for arthritis problem[4], but they do not have scientific validation[5]. Siddha System of medicine basically needs some crude drugs for the preparation of medicine. The crude drugs are derived from different sources such as plants, animals, metals and minerals[6]. Hence, as an alternative, certain plant drugs which are used in Siddha system of medicine could be investigated scientifically to act against arthritis[4]. Therefore, the present study focused on the *in vitro* antioxidant and anti-inflammatory activities of selected herbal drugs such as cardamom (*Elletaria cardamomum* L.), ginger (*Zingiber officinale* Roscoe), arrow root (*Maranta arundinacea* L.), yew leaves (*Abies webbiana* (D. Don) Spach), Indian rose chestnut (*Mesua ferrea* L.), pepper (*Piper nigrum* L) and clove (*Syzygium aromaticum* (L.) Merrill & Perry)[3] to provide scientific evidence for their anti-arthritic effect [4, 5]. The afore-said herbal drugs exhibited therapeutic effect against the loss of appetite, indigestion, gastric reflex, hiccup, flatulence, itching, scabies, cold, cough, head-ache and joint pains[7].

METHODOLOGY

Preparation of the drugs

Raw drugs (*Elletaria cardamomum* L., *Zingiber officinale* Roscoe, *Maranta arundinacea* L., *Abies webbiana* (D. Don) Spach, *Mesua ferrea* L., *Piper nigrum* L., *Syzygium aromaticum* L. Merrill & Perry) were procured from local herbal market, Thanjavur, Tamilnadu, India and identified in the NABL accredited lab of CARISM, SASTRA University and authenticated using macroscopic and microscopic studies[8, 9]. All the drugs were powdered (particle size 1 mm) individually using a lab mill and used for further analysis.

Extract preparation

Ten grams of powdered raw drugs were taken separately in a conical flask and extracted with 100 ml of ethanol and kept for 24 h. Then the extract was filtered through filter

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Table 1: Phytochemical screening results of herbal drugs

S. No.	Phytochemicals	Cardamom um	Ginger	Arrow root	Abies	Mesua	Pepper	clove
1.	Steroids	+	+	-	+	+	-	+
2.	Terpenes	-	+	-	+	-	-	-
3.	Sugars	+	-	-	-	+	+	+
4.	Alkaloids	-	-	-	-	-	+	+
5.	Phenols	-	-	-	+	-	-	+
6.	Flavanoids	+	+	-	+	+	+	-
7.	Tannins	-	+	-	-	-	-	-
8.	Saponins	-	+	-	-	+	+	+
9.	Quinone	-	-	-	-	-	-	+
10	Coumarine	+	+	-	-	+	-	-

Table 2: Total phenolic content of selected herbal drugs

S. No.	Sample Name	Total Phenol content (mg GAE/100 g)
1.	*Elletaria cardamomum*	104.42 ± 1.06
2.	*Zingiber officinale*	179.42 ± 4.36
3.	*Maranta arundinacea*	35.00 ± 1.65
4.	*Abies webbiana*	1314.17 ± 17.68
5.	*Mesua ferrea*	2194.17 ± 515.01
6.	*Piper nigrum*	226.75 ± 19.21
7.	*Syzygium aromaticum*	460.00 ± 49.50

Table 3: Antioxidant activity of selected herbal drugs

S. No.	Sample Name	Antioxidant activity (DPPH assay, %)
1.	*Elletaria cardamomum*	37.53 ± 5.274
2.	*Zingiber officinale*	80.30 ± 3.461
3.	*Maranta arundinacea*	26.69 ± 2.967
4.	*Abies webbiana*	80.94 ± 1.071
5.	*Mesua ferrea*	82.52 ± 0.330
6.	*Piper nigrum*	59.21 ±8.406
7.	*Syzygium aromaticum*	83.33 ± 0.494

Table 4: Anti-inflammatory activity of selected herbal drugs

S. No.	Sample Name	Anti-inflammatory assay
1.	Elletaria cardamomum	89.97 ± 0.964
2.	Zingiber officinale	34.34 ± 0.857
3.	Maranta arundinacea	72.17 ± 0.357
4.	Abies webbiana	83.56 ± 1.750
5.	Mesua ferrea	58.64 ± 4.071
6.	Piper nigrum	85.15 ± 3.857
7.	Syzygium aromaticum	4.70 ± 0.429

paper and used for the analysis.

Phytochemical screening

Phytochemical profile of herbal drugs extract was analyzed as per the methodology of Harborne[10]. For steroids test one ml of extract was taken and add few drops of concentrated sulphuric acid, shake well and kept away some time and noted for colour change. Terpenoids were analyzed by heating the extract in mild flame with tin and thionylchloride and noted the colour change. For alkaloid test, 1 ml of sample was added with 1 ml of diluted acetic acid and few drops of Dragendorff's reagent and noted the precipitate. For the phenol test 0.5 ml of extract was taken with 1 ml of alcoholic ferric chloride and noted for colour change. For flavonoids test, 0.5 ml of extract was taken and 1 mg of magnesium turning and few drops of concentrated hydrochloric acid were added, boiled for 5 min and noted the colour change. For the tannins experiment, few drops of ferric chloride solution was added with extract and noted the colour change. Presence of saponins was examined by taking the extract with water and shaken hardly and noted for froth. For the quinone, 0.5 ml of extract was added with 1 ml of sodium hydroxide (10%) and observed for colour change. For the coumarin assessment 0.5 ml of extract was taken in a test tube with 1 ml NaOH and shaken well and noted the colour change. To check the presence of sugars, extract was treated with Fehlings solution A and B and boiled and noted for precipitation colour.

Total phenol content

The total phenolic concentration of ethanolic extract of all the drugs was estimated according to the modified Folin-Ciocaltue reagent method[11]. Extract (10 µl) was taken in a 96 well microplate and 25 µl of Folin reagent and 230 µl of 4.4% of Na_2CO_3 were added and incubated for 30 min in dark place. Then the absorbance was measured at 750 nm in the ELISA plate reader (Make: Biotek, Model: Epoch). A calibration curve was prepared using standard gallic acid (100 – 1000 mg/L, R^2 = 0.9978) and used to express the results as gallic acid equivalents (GAE).

Antioxidant activity

The antioxidant activity of ethanolic extracts was analyzed using DPPH free radical scavenging assay[12]. Extracts (10 µl) were taken in the 96 well microplates and 200 µl of DPPH solution (2.5 mg/100 ml) and incubated for 30 min in dark place. Then the absorbance was measured at 515 nm in the ELISA plate reader (Make: Biotek, Model: Epoch). The radical scavenging activity of tested samples was calculated using the formula (Antioxidant activity = Abs control – Abs test / Abs control x 100) and expressed on percentage basis.

Anti-inflammatory activity

The anti-inflammatory activity of the ethanolic extracts was evaluated using RBC membrane stabilization method[13]. Blood sample (2 ml) was collected from volunteer in a heparinized tube and washed with PBS twice and centifuged at 3000 rpm for 10 min (Centrifuge Make: Eppendorf, Model 5810-R). Then RBC was suspended in

Table 5: Anti-arthritic and related activities reported in various herbal drugs and their major phytoconstituents.

S. No.	Name of the drug	Action	Compound
1	*Elletaria cardamomum*[30]	Immuno-modulatory	1,8-Cineole
2	*Zingiber officinale*[31]	Analgesic and Anti-inflammation	Gingerol
3	*Maranta arundinacea*[32]	Energy producer and Increase bone strength	Genistein and Daidzein
4	*Abies webbiana*[27]	Anti-inflammation	Aziridine and Abiesin
5	*Mesua ferrea*[33]	Anti arthritic	4-Alkyl- and 4-phenylcoumarins and mesuaferrin A
6	*Piper nigrum*[34]	Antipyretic and Analgesic	Piperine
7	*Syzygium aromaticum*[35]	Antipyretic and Analgesic	Eugenol

normal saline and taken in a tube (0.5 ml) with 0.5 ml of extract and 0.5 ml hypotonic solution and incubated for 30 min at room temperature. Then the contents were centrifuged at 1500 rpm for 10 min and the supernatant was collected and the absorbance was read at 560 nm using Micro plate reader (Make: Biotek, Model: Epoch). Based on the absorbance of extract and control, the membrane stabilization effect was calculated and expressed on percentage basis.

RESULTS AND DISCUSSION

Data obtained in the preliminary phytochemical screening of herbal drugs was shown in Table 1. Red colour was formed when extract was treated with few drops of concentrated sulphuric acid, which shows the presence of sterols in cardomomum, ginger, yew, Mesua and clove. This result was similar to earlier reports in cardomomum[14]. Extract given pink colour upon reaction with tin and thionylchloride in terp**e**noids test in ginger and yew. This same result was noted in previous work[15]. For alkaloid test, orange red precipitate was formed during extract of pepper and clove when treated with acetic acid and few drops of Dragendorff's reagent. Bluish black colour was appeared in phenol test in yew and clove extracts. Presence of flavonoids was noted in cardomomum, ginger, yew, mesua and pepper, based on formation of red colour during extract was reacted with magnesium turning and concentrated hydrochloric acid. Similarly the presence of flavonoid was reported in pepper[16] and yew[17]. Extracts of ginger shows the positive result of bluish black colour when few drops of ferric chloride solution was added. Presence of saponins was noted based on appearance of froth in ginger, mesua, pepper and clove. Similarly the presence of saponins was reported in clove[16]. Quinone was found to be present only in clove based on the observation of end point colour when extract added with sodium hydroxide. Yellow colour was formed when extract was treated with NaOH, which indicated the presence of coumarin in cardomomum, ginger and mesua. This is in agreement with previous result in cardomomum[14]. Presence of sugars was recorded in the extracts of cardomomum, mesua, pepper and clove which was confirmed by the formation of brick red coloured precipitation upon treatment with Fehlings solution A and B. This result was similar to earlier reports in mesua buds[18].

Among the presently investigated herbal drugs *Mesua ferrea* showed the highest total phenolic content (2194.17 mg GAE/100 g), which is followed by *Abies webbiana* (1314.17 mg GAE/100 g). Previous, higher level of total phenol content was noted in *M. ferrea* 98.15mg GAE/g [19] and *A. webbiana*. Phenolic compounds represent the largest group known as 'secondary metabolites' synthesized by higher plants, probably as a result of antioxidative strategies. Polyphenols can scavenge the free radicals such as .OH, O2.-, and ROO. and suppress the free radical-mediated oxidation. The formation of free radicals may be inhibited by reducing hydroperoxides and hydrogen peroxide and by sequestering metal ions through complexation / chelation reactions. The antioxidant and anti-inflammatory role of polyphenols were proven in past studies[20].

Antioxidant activity was determined using 1,1-diphenyl-2-picrylhyrazyl (DPPH) radical method. This assay is based on the measurement of the loss of DPPH colour at 517 nm after reaction with test compounds and the reaction is monitored by a spectrophotometer. DPPH is a stable free radical of purple colour and in the presence of an antioxidant its colour changes to yellow based on the efficiency of the antioxidant. The change in the absorbance with respect to control (DPPH solution, 100 % free radical) is calculated as per cent scavenging power. Radical scavenging action is dependent on both reactivity and concentration of the antioxidant. The antioxidant activity of herbal drugs was measured in terms of DPPH radical scavenging potential and the results are shown in Table 3. Among the drugs, clove (83.33%), Mesua (82.52%), ginger (83.33%) and Abies (80.94%) exhibited maximum level of antioxidant activities. The higher level of antioxidant activity recorded in Mesua and Abies is correlated with their high content polyphenols (Table 2). Similar level of antioxidant activity was reported in previous studies on *Zingiber officinale* (79.0%)[21], *Mesua* (81%)[19] and clove (93%)[22].

Some free radicals such as nitric oxide, superoxide radical anion, and related reactive oxygen species (ROS) and or reactive nitrogen species (RNS) mediate cells in signalling processes[23, 24]. Oxidative stress is an imbalanced state where excessive quantities of ROS/RNS overcome endogenous antioxidant capacity, leading to oxidation of enzymes, proteins, DNA and lipids. Oxidative stress is the contributing feature in the development of chronic degenerative diseases including coronary heart disease,

cancer, arthritis, and aging[25]. The DPPH can be used as a model free radical to resemble the other biological radicals that involved in oxidative stress condition.

Typical inflammatory diseases such as rheumatoid arthritis, asthma, colitis and hepatitis are the leading cause of disability and death. The excessive production of reactive oxygen metabolites by phagocytic leucocytes during the inflammatory process, as part of host defence, disregulates cellular function causing tissue injury which in turn augments the state of inflammation leading to chronic inflammatory diseases. Antioxidants, which scavenge these reactive oxygen metabolites, have been found to complement the anti-inflammatory process and promote tissue repair[26]. In the present study, we have used RBC membrane stabilization assay to evaluate the anti-inflammatory activity of herbal drugs. A possible explanation for the membrane stabilization effect could be an increase in the surface area / volume ration of the cell. The membrane denaturation of RBC was brought about by expansion of cell membrane by incubating the cells in hypotonic solution.

Among the presently studies herbal drugs of cardamomum (89.97%), pepper (85.15%) and Abies (83.56%) were exhibited higher level of anti-inflammatory activities in terms of RBC membrane stabilization. The notable anti-inflammatory activity of Abies could be due to the high amount of polyphenols recorded in the same sample of the present study (Table 1) These results are supported by the previous *in vivo* studies on anti-inflammatory activity of cardomomum, pepper and Abies [27-29].

Based on literature survey, we have found the presence of different phytochemical compounds in selected herbal drugs and their anti-arthritic and related activities (Table 5). So, only the Mesua ferrea exhibits anti-arthritic effect based on literature reference, while ginger, pepper and clove were showed analgesic properties. Both ginger and Abies revealed anti-inflammatory activity whereas cardamom displayed immune-modulatory property. So, consumption of these herbal drugs could relieve the arthritis pain and also strengthen the bone joints in addition improve the immune system and prevent the cellular damages through antioxidant and anti-inflammatory mechanisms.

CONCLUSION

In the present work, the total phenolic content, antioxidant and anti-inflammatory activities of ethanolic extract of different herbal drugs were analyzed. Even though these drugs have been used to treat arthritis in folklore medicine, the mechanism of action is not yet proved and hence the present investigation provides scientific evidence for the action of this drug against arthritis through antioxidant and anti-inflammation pathways. Among the drugs, ginger, Abies, Mesua and clove are responsible for the high antioxidant power while anti-inflammatory property was contributed by cardamom, Abies, Maratha and pepper. So, combination of these drugs could exhibit both antioxidant as well anti-inflammatory activities and thus provides therapeutic effect against arthritis as indicated by the *in vitro* studies. Further *in vivo* models are necessary to prove the efficacy and mechanism of action of this drug against arthritis, which is a major burden for aged people throughout the world.

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