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Investigation of phytochemicals and *in vitro* anti-arthritic activity of methanol extract of *Maesa indica* (Roxb.) leaves

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Abstract
Present investigation aim to open new scientifically established avenues for the improvement of medicinal uses associated with *Maesa indica* leaves, for phytochemical screening and *In-vitro* anti-arthritic activity. After phytochemical screening of the plant leaf extract of *Maesa indica*, there were presence of carbohydrate, reducing sugar, flavonoid, phenol, unsaturated sterol, triterpene and resin. The methanol extract of the plant leaves was assayed for anti-arthritic effect by inhibition of protein denaturation method. In highest concentration *Maesa indica* showed moderate anti-arthritic activity (79.53%) in comparison with Diclofenac -Na. The prime objective of the studies is to establish a cost-efficient and environment friendly anti-arthritic herbal drug to ensure better health care.

Keywords: Phytochemicals, *in vitro*, anti-arthritic, *Maesa indica*, Diclofenac –Na

Introduction
Bioactivity directed research protocol for the authentic medicinal plants is the determination factor for safety, efficacy and quality of herbal medicines [1]. The bioactivity of a medicinal plant is thought to be provided by plant's secondary metabolites. Over the years many research program scientifically established many naturally occurring medicinal plant as dietary supplements, alternative medicines and commercial products [2]. Because of the low therapeutic value and general toxicity level of the synthetic medicines, consumers are diverted towards natural alternatives. Appropriate substitution if systemically analyzed, can establish newly developed natural product which is bio-equivalent to the original one.
Arthritis is a term used that is described as the swelling, pain and inflammation of joints along with other 100 conditions [3]. One-fifth of the world's population is affection by arthritis [4]. It's an autoimmune disease where leukocytes attack body's own cells treating them as antigens. The disease is being categorized as a true auto-immune disease with evidence showing affinity of pathogenic disease-specific autoimmunity towards deiminated proteins. Deimination is termed as the process where amino acid arginine is catalyzed by and enzyme known as PAD. The deiminated proteins mediate inflammation in the joints by forming an immune complex [5]. However, the pathogenesis of the disease is quite complex. Involvement of macrophages, T and B cells, fibro- blasts, chondrocytes and dendritic cells are prominent. Some microbes are evident to trigger the processes of antigen presenting cells, thus triggering the disease. Genetically inherited rouge immunity cells also repeatedly activate the auto-immunity process. As a result cartilage, bone and tendon's composition and structure is damaged and protein is subjected to denaturation. Inflammation occurs as part of body's defense mechanism where blood vessels start to dilate around the damaged area to increase the healing process [6]. Progression of the disease is the determining factor for medication. Joint pain and swelling management relies upon anti-inflammatory and corticosteroid drugs, where auto-immune response is suppressed by disease modifying anti-rheumatic drugs (DMARDs). NSAIDs produce serious side effects like, gastrointestinal bleeding, ulceration, hepatic and renal failure [7]. Certain NSAID drugs may not work against arthritis, as arthritis occurs mostly due to T-cell, B-cell and macrophage related inflammation [8]. Hence, there's an ongoing search for alternative medications from plant and natural sources.
The plant *Maesa indica* belongs to the family of Myrsinaceae. It's a large shrub which is commonly known as Ramjani in Bengali. The plant is inhabited in the hilly areas of south-east Asia. The fruits of the plant have excellent nutritional value and are eaten by the locals of Kotagiri hills [9].

The major content of the plant is quercetin the flavonoid, a phytochemical which is responsible for anti-arthritic and anti-oxidant activity [10]. However, the investigation of phytochemicals and anti-arthritic effect of the plant is still unperformed.

The aim of the studies is to investigate phytohemicals and anti-arthritic activity of methanol extract of *Maesa indica* leaves. The research was performed to evaluate natural alternative for anti-arthritic medication, which will be used as eco-friendly, cost effecient and bio-equivalent herbal drug alongside with the other classes of anti-arthritic medicines.

Material and Method

Plant collection and identification

Leaf of *Maesa indica* was collected from Kaptai, Rangamati, Chittagong, Bangladesh in the month of April 2019. The plant was authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, and Bangladesh.

Preparation of Extract

The leaves of the plant were dried and ground (Moulinex Blender AK-241, Moulinex, France) into powder (40-80 mesh, 500 g) and soaked for 7 days with 2–3 days interval in 2.0 L of methanol at room temperature (23 ± 0.5 °C). Filtrate obtained through cheesecloth and Whatman filter paper No. 1 was concentrated under reduced pressure at the temperature below 50 °C using rotary evaporator (RE 200, Sterling, UK). The extracts (yield 4.4–5.6% W/W) were all placed in glass Petri dishes (90 X 15 mm, Pyrex, Germany).

Preliminary qualitative phytochemical screening

Preliminary qualitative phytochemical screening was carried out according to standard Harbone (1998) method [11]. Secondary plant materials were screened by using simple tests. The tests were performed for alkaloids, carbohydrate, glycoside, reducing sugar, flavonoid, phenol, tannin, unsaturated sterol, saponin, triterpene and resin in the sample. The methods used were described by Harbone (1998) except otherwise stated. Test results were expressed as present (+), moderately present (++), readily present (+++), absent (-).

Test for Alkaloids

200 mg of extract was taken and diluted into 10 ml with Methanol, which was later boiled and filtered. In 5 mL of filtrate, added 2 ml of dilute Ammonia, 5 ml of Chloroform and shaken gently to extract the alkaloidal base. After that the chloroform layer was extracted with 1 ml of Acetic Acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent was added to another portion. The formation of a cream yellow (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) indicates positive for alkaloids.

Test for Carbohydrates

0.1 g extract was boiled with 2 ml of distilled water and it was filtered. Few drops of naphthol solution in ethanol (molisch's reagent) were added into the filtrate. Concentrated H_2SO_4 in a Pasteur pipette was poured down with ease on the side of the test tube which forms a lower layer. A purple ring in the middle indicates the confirmation of carbohydrate.

Test for Glycosides

5 ml dilute H_2SO_4 was added to 0.1 g of plant extract in a test tube and the mixture wasboiled for 15 min in a water bath, then allowed to be cooled and neutralized with 20% potassium hydroxide solution. 10 ml of a mixture of equal parts of Fehling's solution A and B was added to the mixture and boiled for 5 min. Dense brick red precipitate indicates the presence of glycoside.

Test for Reducing sugar

5 ml mixture of equally distributed parts of Fehling's A and B solution was added to 5 ml of extract. Then the mixture is heated in a water bath for 5 min. Brick red precipitate shows the confirmation of reducing sugar.

Test for Flavonoids

Small amount of the extract was boiled in 10 ml ethyl acetate for 3 minutes which was later filtered and allowed to cool. 4 ml filtrate was shaken with 1ml of dilute NH_3 solution. Appearance of an intense yellow colour indicates the presence of flavonoids.

Test for Saponins

5 ml aliquot of the extract was diluted with 20ml of deionized water which was shaken vigorously and observed. Persistent froth indicates the presence of saponins.

Test for Tannin

1 ml extract solution was added to 10 ml of deionised water and then treated with 3 drops of $FeCl_3$. A greenish-brown precipitate indicated the confirmation of tannins.

Test for Unsaturated sterols and Triterpene

10 mg extract was taken and added in 1 ml of chloroform. 1 ml acetic anhydride followed by 2 ml concentration H_2SO_4 was added to the mixture. Formation of reddish violet color represents the presence of triterpene and unsaturated sterol.

Test for Resin

Take 5ml 10% ethanolic extract solution. Boiled for 20 min and filtered and 5ml DH2O added. Precipitation indicates resin presence

Test for Phenol

0.1 gm of plant extract was added to 10 ml distilled water. The solution was heated in thr water bath for 3 min and it was filtered. 2 ml of the filtrate was placed in each of the 3 test tubes. The filtrate in one of the test tubes was diluted with distilled water in the ratio 1:4. Appearance of blue or greenish color indicated the presence of phenols.

In vitro anti-arthritic activity

For the evaluation *in vitro* anti-arthritic activity of *Maesa indica* "inhibition of protein denaturation" method was used using Diclofenac-Na as standard [12-13]. The test solution (0.5 ml) Consists of 0.45 ml bovine serum albumin (5% w/v) and 0.05 ml of test solution methanol extract of *Maesa indica* at various concentration. The test control (0.5 ml) is prepared by adding 0.45 ml of bovine serum albumin (5% w/v) with 0.05 ml distilled water. The product control (0.5 ml) consists 0.45 ml distilled water and 0.05 ml of test solution. Standard solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (BSA) and 0.05 ml of Diclofenac-Na at different doses. 0.05ml various concentration of test drugs (62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500µg/ml, 1000 µg/ml) and standard drug at different concentration (62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500µg/ml, 1000 µg/ml) were taken respectively and 0.45 ml BSA 5% mixed. All the solutions in the test tubes were adjusted to pH 6.3 using 1 N HCl. Sample was incubated at

37 degree for 20 minutes. After that the temperature was increased and kept the sample at 57 degree for 3 minutes. After cooling down the solutions 2.5 ml phosphate buffer was added with above solution. Absorbance was taken in UV spectrophotometer at 416 nm. The inhibition percentage was calculated. Percent of inhibition of protein denaturation is calculated as follows:

Percentage Inhibition = 100 – [{(absorbance of test solution – absorbance of product control) ÷ (absorbance of test control)} × 100]

Control represents 100% protein denaturation. The result was compared with standard anti-arthritic agent Diclofenac-Na.

Statistical analysis

The data was analyzed statistically using ANOVA followed by student't' test with GraphPad Prism Data Editor for Windows, Version 6.0 (GraphPad software Inc., San Diego, CA). Values were expressed as mean ± Standard error for mean (± SEM). $P<0.05$ - 0.01 were considered as statistically significant.

Results

Phytochemical screening

The result obtained in the present investigation (Table-1), the methanol extract of the leaves of *Maesa indica* showed the presence of Carbohydrate, Reducing sugar, Flavonoid, Phenol, Unsaturated sterol, Triterpene and Resin.

Table 1: Analysis of phytochemicals in the methanol extract of *Maesa indica*

Number	Phytochemicals	Presence
1	Carbohydrate	+
2	Glycoside	-
3	Reducing sugar	+
4	Flavonoid	+
5	Phenol	+++
6	Tannin	-
7	Unsaturated sterol	++
8	Triterpene	++
9	Saponin	-
10	Resin	+
11	Alkaloid	-

***In vitro* anti-arthritic activity**

Denaturation of protein, lysis of membrane or protein action may be the underlying cause of auto antigen production in some kind of arthritis. The maximum percentage inhibition of protein denaturation membrane stabilization and proteinase inhibitory action were observed as *M. indica* (79.53%, 67.25%, 63.15%, 57.30%, 49.70%) at 400, 200, 100, 50, 25 µg/ml respectively as shown in Table 2. From the results shown in Figure 1, our study reveals that methanol extract of *M. indica* has the capability to control the production of auto antigen and inhibits protein denaturation, membrane lysis and proteinase action in rheumatic disease.

Table 1: Analysis of *in vitro* anti-arthritic activity in the methanol extract of *Maesa indica*

Concentration (µg/ml)	% of protein denaturation inhibition of methanol extract of *Maesa indica* Mean ± SEM	% of protein denaturation inhibition of Diclofenac-Na Mean ± SEM
400	79.53 ±2.12	94.16±2.27
200	67.25±1.32	92.25±1.17
100	63.15±2.22	88.19±3.31
50	57.30±0.83	81.35±2.31
25	49.70±1.47	76.49±1.23

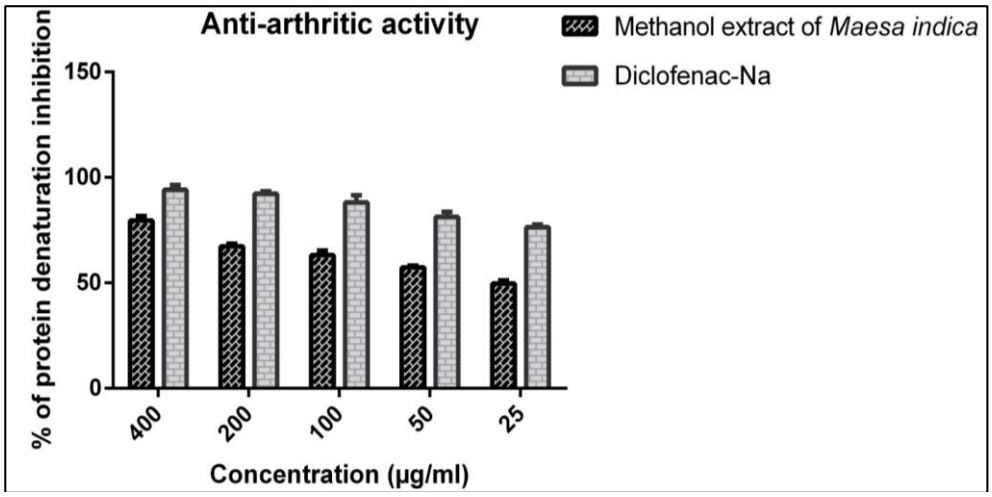

Fig 1: Percentage inhibition of protein denaturation

Discussion

A variety of phytochemicals present in the herbs and herbal plants have significant therapeutic index. Different phytoconstituents have been reported to perform a wide variety of functions, which may help against chronic diseases with safer therapeutic value. For instance steroids, terpenoids, flavanoids, phenols and tannins acquired from various medicinal plants are reported as anti-arthritic agents [14]. Phytochemical investigation was performed to set forward a guideline for further therapeutic evaluation for *Maesa indica* in the future. According to the investigation the plant contains some major group of phytochemicals such as carbohydrate, reducing sugar, flavonoid, phenol, unsaturated sterol, triterpene and resin. The outcome further ensures the scenario of the plant having the probability of possessing anti-arthritic effect.

Denaturation of tissue protein is one of the reported causes of inflammatory and arthritic diseases. *In vivo* denaturation of proteins may be the underlying factor for the production of auto-antigens in certain arthritic diseases. The mechanism is probably due to the alteration of I electrostatic hydrogen, hydrophobic and disulphide bonds. The increments in absorbance of plant extract and reference drug in comparison with control indicated the stabilization of albumin protein. This anti-denaturation effect was further supported by the change in viscosities [15-16]. It has been documented that the viscosities of protein solutions rise on denaturation. Allocated numbers of drug are available as market preparation as a part of the treatment of arthritis. But all of the classes are noted to demonstrate side effects and toxicity. Among these classes NSAIDs show side effects related to gastritis, ulceration and high blood pressure due to inhibition of prostaglandin. Long time use of NSAID can produce hepatic and renal failure [17]. Where corticosteroids show side effects related to weight gain, diabetes and osteoporosis [18]. It is also reported to mildly suppress hypothalamic-pituitary axis, to severe life-threatening infection [19]. On the other hand side effects of DMARDs are generally stomach upset, liver and blood problem. Sometimes the side effect of stomach upset is so severe that it becomes intolerable to certain patients [20]. These classes of drugs also function in various procedures. NSAIDs and corticosteroids function by inhibiting inflammatory mediators where DMARDs work by modifying T-cell, B-cell, IL-1 or other immunity cells [21]. The anti-arthritic effect presented by the plant extract could be due to any mechanism of the above. Further specific studies with more improved research facilities are needed to determine the underlying mechanism.

Conclusion

In spite of tremendous development in the sector of medicine now days, they possess various side effects, whereas plants still uphold their own unique place, being a natural product which shows no side effects and are well tolerated. Therefore, a systematic approach should be taken to determine the potency of plants against arthritis, so as to exploit them as herbal anti-arthritic agent.

According to the investigation, the plant contains some major group of phytochemicals. In inhibition of protein denaturation method, *Maesa indica* extract showed dose depended inhibition of protein denaturation throughout the dose range from low to high. Increase in the viscosities further supported this anti-denaturation effect. The finding of *in vitro* model suggested that methanol extract of *Maesa indica* has moderate anti-arthritic potential. In future isolation of lead molecules responsible for the activity will be carried out which may be beneficial for the development of new anti-arthritic agent.

Conflict of Interests: The authors declare that there is no conflict of interest regarding the publication of this paper

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