

Evaluation of analgesic , anti-inflammatory and CNS activities of the methanolic extract of *Syzygium Samarangense* bark.

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ABSTRACT : *Syzygium Samarangense* (red jamrul in Bangladesh) is a potential medicinal drug. We aimed to evaluate the analgesic, anti-inflammatory and CNS activities of the methanolic extract of *Syzygium Samarangense* bark in mice. The analgesic activity was examined by acetic acid induced writhing and formalin tests. The anti-inflammatory activity was studied using carrageenan induced hind paw edema model. The analgesic activity of the methanolic extract of *Syzygium Samarangense* bark was evaluated by acetic acid induced writhing and formalin tests at the dose of 100 mg/kg and 200 mg/kg, significantly ($p < 0.05$) reduced the writhing caused by acetic acid and the number of licks induced by formalin in a dose dependent manner. The extract of *Syzygium Samarangense* bark caused significant ($p < 0.05$) inhibition of carrageenan induced paw edema after 4 h in a dose dependent manner. The CNS depressant activity was evaluated by observing the reduction of locomotor and exploratory activities in the open field and hole cross tests at a dose of 100 mg/kg and 200 mg/kg body weight. The findings of the study suggested that the methanolic extract of *Syzygium Samarangense* bark has strong analgesic, moderate effect against inflammation and significant CNS effects, conforming the traditional use of this plant for inflammatory pain alleviation.

KEYWORDS: Analgesic, Anti-inflammatory, Bark, CNS activities, Methanolic extract of *Syzygium Samarangense*, Pain

I. INTRODUCTION

The use of the medicinal plants is increasing in many countries where 35% of drugs contain natural products. At present, thousands of plant metabolites are being successfully used for the treatment of variety of diseases. The investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant based drugs. In Bangladesh thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. The beneficial medicinal effects of plant materials typically results from combinations of secondary product present in plant such as alkaloids, steroids, tannins, phenol compounds, resins, gums, flavonoids and fatty acids which are capable of producing definite physiological action on body.

Syzygium samarangense belongs to the Myrtaceae family and is among the fruits of economic importance in Asia and Taiwan. Common names of *Syzygium samarangense* are wax apple, love apple, java apple, chomphu (in Thai), Man (in Vietnam), bellfruit (In Taiwan), Jamaican Apple, Otaheti Apple (in Jamaica), jambu air (in Indonesian), water apple, mountain apple, cloud apple, jambu air ("water guava" in Malay), wax jambu, rose apple, bell offruit, makopa, tambis (Philippines), and chambekka in Malayalam, jamrul (in Bengali), and jumbu (Sri Lanka). *Syzygium samarangense* is a tropical tree growing to 12 m tall, with evergreen leaves 10–25 cm long and 5–10 cm broad. The flowers are white, 2.5 cm diameter, with four petals and numerous stamens. The fruit is a bell-shaped, edible berry, with colors ranging from white, to red or green to red, purple, or crimson, to deep purple 4–6 cm long in wild plants. The flowers and resulting fruit are not limited to the axils of the leaves, and can appear on nearly any point on the surface of the trunk and branches. When mature, the tree is considered a heavy bearer, yielding a crop of up to 700 fruits.

Its leaves, roots, bark, and fruit all have potential medical applications. Some Myrtaceae plants have reportedly been used as medicinal herbs for the treatment of bronchitis, asthma, diabetic mellitus, and inflammation syndromes. They exert potent free radical scavenging, antioxidation, antimitutation, and anticancer activities. Previous study identified that extracts from guava (*Psidium guajava* L.) leaves displayed antihyperglycemic ability in Type 2 diabetic mice and recognized quercetin as the major active component in the extracts. The leaves of wax apple also contain abundant phytochemicals, including ellagitannins, flavanones,

flavonol glycosides, proanthocyanidins, anthocyanidins, triterpenoids, chalcones, and volatile terpenoids. Wax apple fruit has reportedly demonstrated antihyperglycemic activity in alloxan-induced (Type 1 DM) diabetic mice. *Syzygium samarangense* invites great attention for researchers worldwide due to its various pharmacological activities such as anti-diabetic, antimicrobial activity, antiviral activity, spasmolytic activity, protease inhibitory/intiamnesiac activity, immunomodulatory activity, antihyperglycaemic activity, analgesic and antiinflammatory activities [Edible Medicinal And Non-Medicinal Plants: Volume 3, Fruits] and woundhealing. The red fruits are juicier and more flavorful and suitable for eating out-of-hand. In Malaya, the greenish fruits are eaten raw with salt or may be cooked as a sauce. They are also stewed with true apples. The flowers are astringent and used in Taiwan to treat fever and halt diarrhea. The phytochemicals in the Java apple tree show some antibiotic action against staphylococcus aureus, candida albicans and mycobacter smegmatis.

Investigators have found the flowers principal constituent to be tannin. The red, hard wood is used for constructing huts in the Andaman and Nicobar Islands.

The fruits are used in traditional medicine to cure diabetes. Several *syzygium* species are reported to possess antibacterial, antifungal and anti-inflammatory activities. The flavonoids isolated from *S. samarangense* were reported to possess antihyperglycemic activity, spasmolytic activity and immunomodulatory activity. Fractionation of the methanolic extracts of the pulp and seeds of the fruits of *Syzygium samarangense* led to the identification of four cytotoxic chalcones and eight antioxidants. Prolyl endopeptidase inhibitors were isolated from the hexane extract of the leaves of *S. samarangense*. A new triterpene methyl-3-epi-betulinic acid in its native form and 4', 6'-dihydroxy-2'-methoxy-3', 5'-dimethyl chalcone along with ursolic acid, jacoumaric acid and arjunolic acid have been isolated from the aerial parts of *Syzygium samarangense*.

II. MATERIALS AND METHOD

2.1 Animal selection:

Swiss albino mice (25-30g) were used for assessing biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water *ad libitum*. The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into different groups, each consisting of five animals which were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee, Atish Dipankar University of Science & Technology, Dhaka, Bangladesh. Animal treatment and maintenance for acute toxicity and analgesic effects were conducted in accordance with the Principle of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Atish Dipankar University of Science & Technology, Dhaka, Bangladesh.

2.2 Collection of Plant materials

The bark of the *Syzygium Samarangense* (red jamrul) were collected from the adjoining area of Jahangirnagar University Campus, Bangladesh during April 2013. The plant material was taxonomically identified by the National Herbarium of Bangladesh-Dhaka.

2.3 Chemicals

Diclofenac Na, Ibuprofen and Diazepam were obtained from Square Pharmaceuticals Ltd., Bangladesh, Acetic acid was collected from Merck, Germany. Normal saline water (0.9%) NaCl was brought from Beximco Infusion Ltd. Bangladesh. Formalin, carageenan and all other chemicals were of analytical grade.

2.4 Preparation of plant extract

The plant material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container. The dried powder material (1.2 kg) was refluxed with methanol for three hours. The total filtrate was concentrated to dryness, *in vacuo* at 40°C to render the methanol extract (310 g). The obtained methanolic extract was concentrated reddish black colored. The extract was preserved in an air-tight container as a crude extract sample.

2.5 Acute toxicity study

Acute oral toxicity assay was performed in healthy nulliparous and non pregnant adult female albino Swiss mice (25-30g) divided into different groups. The test was performed using increasing oral dose of the methanolic extract of *Syzygium Samarangense* bark in water (50, 100, 200, 500, 1000 mg/kg body weight), in 20 ml/kg volume to different test groups. Normal group received water. The mice were allowed to feed *ad libitum*, kept under regular observation for 48 h, for any mortality or behavioral changes (Sanmugapriya and Venkataraman, 2006).

2.6 Analgesic activity:

2.6.1 Acetic acid-induced writhing test:

The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. The animals were divided into six groups with five mice in each group. Group I animals received vehicle (water), Group II received Diclofenac Na at 10 mg/kg body weight while animals of Group III and IV were treated with 100 mg/kg and 200 mg/kg body weight (p.o./per oral) of the methanolic extract of *Syzygium Samarangense* bark.. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 1.0% v/v acetic acid but Diclofenac-Na was administered intraperitoneally 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min (Ahmed *et al.*, 2004).

2.6.2 Formalin test

The antinociceptive activity of the drugs was determined using the formalin test described by Dubuission and Dennis (1977). Control group received 5% formalin. 20 µl of 5% formalin was injected into the dorsal surface of the right hind paw 60 min after administration of the extract of *Syzygium Samarangense* bark (100 mg/kg and 200 mg/kg, per oral) and 30 min after administration of Diclofenac Na (10 mg/kg, i.p./intraperitoneal). The mice were observed for 30 min after the injection of formalin, and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early phase and the period between 15 and 30 min as the late phase. The total time spent licking or biting the injured paw (pain behavior) was measured with a stop watch.

2.7 Anti-inflammatory activity

Carrageenan induced paw edema test in mice

Swiss albino mice (25-30g) were divided into six groups of five animals each. The test groups received 100 mg/kg and 200 mg/kg body weight, (p.o./per oral) of the methanolic extract of *Syzygium Samarangense* bark. The reference group received Ibuprofen (10 mg/kg body weight, per oral) while the control group received 1 ml/kg body weight normal saline. After 1 h, 0.1 ml, 1% carrageenan suspension in normal saline was injected into the subplanatar tissue of the right hind paw. The paw volume was measured at 1, 2, 3 and 4 h after carrageenan injection using a micrometer screw gauge. The percentage inhibition of the inflammation was calculated from the formula:

$$\% \text{ inhibition} = (1 - D_t/D_o) \times 100$$

Where, D_o was the average inflammation (hind paw edema) of the control group of mice at a given time, D_t was the average inflammation of the drug treated mice at the same time (Winter *et al.*, 1962).

2.8 Statistical analysis

All values were expressed as the mean \pm SEM of three replicate experiments. The analysis was performed by using SPSS statistical package for WINDOWS (version 16.0; SPSS Inc, Chicago). Results related to the reducing power activities were statistically analyzed by applying the Student *t*-test and $p < 0.001$ were considered to be statistically significant. All data are subjected to ANOVA followed by Dunnett's test and $p < 0.05$ were considered to be statistically significant.

III. RESULTS

Acute Toxicity Studies

The acute toxicity studies mainly aim at establishing the therapeutic index, i.e., the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species. The methanolic extract of *Syzygium Samarangense* bark was safe up to a dose of 1000 mg/kg (p.o.) body weight. Behavior of the animals was closely observed for the first 3h then at an interval of every 4h during the next 48h. The methanolic extract did not cause mortality in mice during 48h observation but little behavioral changes, locomotor ataxia, diarrhea and weight loss were observed. Food and water intake had no significant difference among the group studied.

In vivo analgesic activity

Acetic acid-induced writhing test

Table 1. shows the effects of both extract of on acetic acid-induced writhing in mice. The oral administration of the methanolic extract of *Syzygium Samarangense* bark significantly ($p < 0.05$) inhibited writhing response induced by acetic acid in a dose dependent manner.

Formalin test

The methanolic extract of *Syzygium Samarangense* bark (100 mg/kg and 200 mg/kg, p.o.) significantly ($P<0.05$) suppressed the licking activity in either phase of the formalin-induced pain in mice in a dose dependent manner (Table 2). The methanolic extract of *Syzygium Samarangense* bark at the dose of 200 mg/kg body weight, showed the almost similar licking activity against both phases of formalin-induced pain than that of the standard drug diclofenac Na.

Anti-inflammatory activity

Carrageenan induced paw edema test

Table 3 showed that the anti-edematous effects of orally administered methanolic extract of *Syzygium Samarangense* bark on carrageenan induced paw edema in mice- a dose dependent moderate type of anti-inflammatory activity but statistically significant ($P<0.05$). Methanolic extract of *Syzygium samarangens* bark showed anti-inflammatory effects at 200 mg/kg dose (59.82% of inhibition), whereas standard diclofenac showed (84.18% of inhibition) of paw edema.

IV. DISCUSSION

Acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics and represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from the tissue phospholipid (Ribeiro *et al.*, 2000). The response is thought to be mediated by peritoneal mast cells (Voilley, 2004), acid sensing ion channels (Hossain *et al.*, 2006) and the prostaglandin pathways (Adzu *et al.*, 2003). The organic acid has also been postulated to act indirectly by inducing the release of endogenous mediators, which stimulates the nociceptive neurons that are sensitive to NSAIDs and narcotics (Alam *et al.*, 2012). It is well known that non-steroidal anti-inflammatory and analgesic drugs mitigate the inflammatory pain by inhibiting the formation of pain mediators at the peripheral target sites where prostaglandins and bradykinin are proposed to play a significant role in the pain process (Kim *et al.*, 2004). Therefore, it is likely that the methanolic extract of *Syzygium samarangens* bark might have exerted its peripheral antinociceptive action by interfering with the local reaction caused by the irritant or by inhibiting the synthesis, release and/or antagonizing the action of pain mediators at the target sites. The above findings clearly demonstrated that both central and peripheral mechanisms are involved in the antinociceptive action of the methanolic extract of *Syzygium samarangens* bark. Interestingly, compounds like flavonoids and steroids, triterpenes in part, have been shown to possess anti-inflammatory, analgesic activity and the claim made by Pritam *et al.* (2011).

The formalin model normally postulates the site and the mechanism of action of the analgesic. This biphasic model is represented by neurogenic (0-5 min) and inflammatory pain (15-30 min), respectively (Hunskar and Hole, 1987). Drugs that act primarily on the central nervous system such as narcotics inhibit both as steroids and NSAIDs suppress mainly the late phase (Alam *et al.*, 2012). The suppression of neurogenic and inflammatory pains by the extract might imply that it contains active analgesic principle that may be acting both centrally and peripherally. This is an indication that the extract can be used to manage acute as well as chronic pain. The mechanism by which formalin triggers C-fibers activation remained unknown for a relatively long time. Recently, however, McNamara *et al.*, (2007) demonstrated that formalin activates primary afferent neurons through a specific and direct on TRPA1, a member of the transient receptor potential family of cation channels, expressed by a subset of C-fiber nociceptors, and this effect is accompanied by increased influx of Ca^{2+} ions. TRPA1 cation channels at primary sensory terminals were also reported to mediate noxious mechanical stimuli (Kerstein *et al.*, 2009). These experiments suggest that Ca^{2+} mobilization through TRPA1 cation channels is concomitant with noxious chemicals and mechanical stimuli as they produce their analgesic action. It is likely that the inhibitory effect methanolic extract of *Syzygium samarangens* bark to pain response is due to inhibit the increase of the intracellular Ca^{2+} through TRPA1, presumably evoked by formalin. So, the extract of *Syzygium Samarangense* bark may contain substances that affect the metabolism of Ca^{2+} .

Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Antonio and Brito, 1998; Gupta *et al.*, 2006; Sawadogo *et al.*, 2006). Since the extract significantly inhibited paw edema induced by carrageenan in the second phase and this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract and this effect is similar to that produced by non-steroidal anti-inflammatory drugs such as ibuprofen, whose mechanism of action is inhibition of the cyclooxygenase enzyme. Flavonoids and saponins are well known for

their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation (Pin *et al.*, 2010). This hypothesis is strongly supported by the previous study, which has shown that methanolic extract of *Syzygium samarangense* bark possess anti-inflammatory activity due to the presence of flavonoid content (Koblyakov, 2001; Vaghasiya *et al.*, 2007).

CNS depressant activity

Hole Cross Test

The method used was done as described by Takagi *et al.* The animals were divided into control, standard and test groups ($n = 5$ per group). The control group received vehicle (water) whereas the test group received methanolic extract of *Syzygium samarangense* bark (at the doses of 100mg/kg and 200 mg/kg p.o.) and standard group received diazepam at the dose of 1mg/kg body weight orally. Each animal was then placed on one side of the chamber and the number of passages of each animal through the hole from one chamber to the other was recorded for 30 min on 0, 30, 60 and 90 mins.during the study period.

Open Field Test

This experiment was carried out as described by Gupta *et al.*, The animals were divided into control, standard and test groups ($n = 5$ per group). The control group received vehicle (water), the test group received the crude extract (at the doses of 100mg/kg and 200 mg/kg p.o.) and standard group received diazepam at the dose of 1mg/kg body weight orally. The animals were placed on the floor of an open field (100 cm×100 cm×40 cm h) divided into a series of squares. The number of squares visited by each animal was counted for 3 min on 0, 30, 60 and 90 mins. during the study period.

RESULT of CNS depressant activity

Open-field test

In the open-field test the methanolic extract of *Syzygium Samarangense* bark extract exhibited a decrease in the movements of the test animals at all dose levels tested. The results were statistically significant for all doses and followed a dose-dependent response (Table 5).

Hole-cross test

Results of the hole-cross test followed a similar trend to the ones observed in the open-field test. They were statistically significant for all dose levels and followed a dose-dependent response. The depressing effect was most intense during the third (60 mins) observation periods (Table 4).

V. CONCLUSION

The results of the experiments suggest that the methanolic extract of *Syzygium Samarangense* bark may be used as an alternative or supplementary herbal remedy for the treatment of analgesic, inflammatory and in depressant disease. Because of its analgesic, anti-inflammatory and antidepressant effects, the methanolic extract of *Syzygium Samarangense* bark have beneficial effects together with drugs known for a strong analgesic, moderate anti-inflammatory as well as antidepressant effects. Thus the present study warrants further investigation involving components of the methanolic extract of *Syzygium Samarangense* bark for possible development of new class of analgesic and anti-inflammatory drugs.

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Table 1: Effects of the methanolic extract of *Syzygium samarangens* bark on acetic acid-induced writhing in mice

Groups	Dose (mg/kg)	No. of writhing	% inhibition
Group I	Vehicle	35.60	-
Group II	10	10.10	71.62*
Group III	100	19.40	45.50*
Group IV	200	12.14	65.89*

Values are mean \pm SEM, (n = 5); *p<0.05 as compared to vehicle control (One way ANOVA followed by Dunnett test). Group I animals received vehicle (water) Group II received Diclofenac Na (10 mg/kg body weight) Group III, IV were treated with 100mg/kg and 200 mg/kg body weight (p.o.) of the methanolic extract of *Syzygium samarangens* bark.

Table 2: Effects of the methanolic extract of *Syzygium samarangens* bark hindpaw licking in the formalin test in mice

Groups	Dose (mg/kg)	Early phase (Sec)	% protection	Late phase (Sec)	% protection
Group-I	Vehicle	36.66 \pm 1.38	-	42.18 \pm 1.03	-
Group-II	10	16.03 \pm 0.90*	56.27	17.23 \pm 0.70*	59.15
Group-III	100	19.0 \pm 2.2*	45.45	15.0 \pm 2.00*	57.39
Group-IV	200	10.0 \pm 3.3*	70.45	9.00 \pm 2.7*	73.30

Values are mean \pm SEM, (n = 5); *p<0.05 as compared to vehicle control (One way ANOVA followed by Dunnet test). Group I animals received vehicle (water) Group II received Diclofenac Na (10 mg/kg body weight) Group III, IV were treated with 100mg/kg and 200 mg/kg body weight (p.o./per oral) of the methanolic extract of *Syzygium samarangens* bark.

Table 3: Effect of methanolic extract of *Syzygium samarengense* in carageenan induced inflammation.

Group	Dose (mg/kg)	Oedema diameter (mm)				Inhibition (%)			
		1h	2h	3h	4h	1h	2h	3h	4h
Group I	Vehicle	5.4 \pm 0.20	6.35 \pm 0.49	6.06 \pm 0.58	6.3 \pm 0.84				
Group II	10	2.4 \pm 0.47*	1.86 \pm 0.46*	1.44 \pm 0.3*	1.0 \pm 0.32*	55.33	70.72	76.25	84.18
Group III	100	4.5 \pm 0.68*	4.2 \pm 0.61*	3.7 \pm 0.51*	3.2 \pm 0.49*	17.68	34.38	38.96	50.02
Group IV	200	3.8 \pm 0.50*	3.62 \pm 0.56*	3.4 \pm 0.46*	2.54 \pm 0.57*	29.86	43.01	46.55	59.82

Values are mean \pm SEM, (n = 5); * p<0.05, Dunnet test as compared to vehicle control. Group I animals received vehicle (water), Group II received Ibuprofen 10 mg/kg body weight, Group III and Group IV were treated with 100mg/kg and 200 mg/kg body weight (p.o.) of the methanolic extract of *Syzygium samarangens* bark.

Table 4. Effect of methanolic extract of *Syzygium samarangens* bark on hole cross test in mice

Group	Dose	Number of Movements				
		0 min	30 min	60 min	90 min	120 min
Group-I	10ml/kg,	118.4 \pm 1.20	118 \pm 1.30	115.4 \pm 0.50	117.4 \pm 1.16	-
Group-II	1mg/kg,	117.2 \pm 1.15*	64.6 \pm 0.43*	38.8 \pm 0.58*	15.8 \pm .86*	-
Group-III	100 mg/kg	3 \pm 1.00*	3 \pm 1.61*	1.8 \pm 0.91*	2 \pm 1.11*	-
Group-IV	200 mg/kg	3 \pm 1.3*	4 \pm 1.7*	2.0 \pm 1.2*	3 \pm 1.2*	-

Values are mean \pm SEM, (n = 6); * p<0.05, Dunnet test as compared to vehicle control. Group I animals received vehicle (water), Group II received diazepam 1 mg/kg body weight, Group III and Group IV were treated with 100mg/kg and 200 mg/kg body weight (p.o.) of the methanolic extract of *Syzygium samarangens* bark.

Table 5. Effect of methanolic extract of *Syzygium samarangens* bark on Open Field test in mice

Group	Dose	Number of Movements				
		0 min	30 min	60 min	90 min	120 min
Group-I	10ml/kg	12.8 \pm 1.15	13 \pm 1.41	13.6 \pm 0.92	14.2 \pm 0.86	-
Group-II	1mg/kg	11.2 \pm 0.58	6 \pm 0.70*	4.0 \pm 0.83*	2.4 \pm 0.81*	-
Group-III	100 mg/kg,	194 \pm 7.00	56 \pm 4.49*	49 \pm 2.15*	38 \pm 4.05*	-
Group-IV	200 mg/kg,	146.2 \pm 7.4	85 \pm 2.7*	48 \pm 3.4*	29 \pm 3.5*	-

Values are mean \pm SEM, (n = 5); * p<0.05, Dunnet test as compared to vehicle control. Group I animals received vehicle (water), Group II received diazepam 1 mg/kg body weight, Group III and Group IV were treated with 100 mg/kg and 200 mg/kg body weight (p.o./per oral) of the methanolic extract of *Syzygium samarangens* bark.