



E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(6): 45-52
Received: 19-09-2020
Accepted: 21-10-2020

Rozina Parul

Senior Lecturer, Department of
Pharmacy, Gono
Bishwabidyalay, Mirzanagor,
Savar, Dhaka, Bangladesh

Ananta Kumar Das

Assistant Lecturer
Department of Pharmacy, Gono
Bishwabidyalay, Mirzanagor,
Savar, Dhaka, Bangladesh.

Md. Sohel Rana

Professor, Department of
Pharmacy, Jahangirnagar
University Savar, Dhaka,
Bangladesh

Comparative pharmacological evaluation in respect to non-polar and polar solvent extracts of the leaves of *Syzygium balsameum* & *Syzygium formosum*

Rozina Parul, Ananta Kumar Das and Md. Sohel Rana

Abstract

The present study was concerned with the evaluation of the medicinal value of the leaves of *Syzygium balsameum* & *Syzygium formosum*. The leaves of the *S. balsameum* and *S. formosum* were successively extracted with hexane, ethyl acetate, and methanol and designated as SBH, SBEa & SBM for *S. balsameum* and SFH, SFEa & SFM for *S. formosum*. Preliminary phytochemical screening of all the extracts revealed the presence of various phytochemical constituents. Evaluation of antioxidant capacity by DPPH method SBM showed more active than standard ascorbic acid. SFM also showed good scavenging activity compare to SBH, SBEa, SFH, SFEa. Total phenolic and flavonoids content of *S. balsameum* & *S. formosum* methanolic extracts exhibited more content compare to hexane & ethyl acetate extract. The total antioxidant capacity of all the extracts showed significant result. In case of cytotoxicity test all the extracts showed mild cytotoxic activity compare to STD. The *S. balsameum* showed more cytotoxic than *S. formosum*. The antimicrobial study of all the extracts showed moderate activity against different organisms but exhibit significant activity against *Bacillus Cereus* & *Bacillus subtilis*. All the extracts showed mild thrombolytic activity. The OGTT showed insignificant activity of hexane and ethyl acetate extracts compared to standard but methanol extract exhibited significant activity compared to control and standard. The results of castor oil and magnesium sulfate-induced diarrhea showed that, statistically insignificant reduction in the incidence and severity of diarrhea with a higher dose of all the extract. The finding of this study suggested that methanol extracts showed more pharmacological activity than others.

Keywords: *Syzygium balsameum*, *syzygium formosum*, phytochemicals, antioxidant, cytotoxicity, antimicrobial.

Introduction

Nature is always a golden sign to show the prominent phenomena of coexistence. Natural products from plants, animals, and minerals are the basis for treating human diseases [1]. Natural products and their derivatives have been recognized for many years as a source of therapeutic agents and structural diversity. Natural products have a wide range of diversity of multidimensional chemical structures and the utility of natural products as biological function modifiers [2]. Medicinal plants have undoubtedly been considered by human beings since ancient times. In ancient Persia, plants were commonly used as a drug and disinfectant and aromatic agent [3]. The use of medicinal plants for the treatment of diseases dates back to the history of human life, that is, since human beings, the use of plants was their only choice of treatment. [4] In traditional methods, plant materials are tested for pharmaceutical purposes. The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally called medicinal plants. A large number of important modern drugs and most of the traditional medicines are derived from medicinal plants. Some secondary metabolites of plants and animal origins like alkaloids, glycosides, flavonoids, and polyphenols possess medicinal properties [4]. Therefore, the rationality of the present study lies in meeting the developing medicines from natural sources. That's why the present study is aims to investigate different fractions and evaluate the biological properties of *Syzygium balsameum* and *Syzygium Formosum*. A good amount of research work has already been conducted on different species of *Syzygium*. However, literature about the plants *Syzygium balsameum* (Buti-jam) and *Syzygium formosum* (*Paniya Jam*) species doesn't show any significant research. A scientific study has found anti-allergic effects of the ethanol extract of *Syzygium formosum* (Wall.) Masam leaves and their immunoregulatory mechanisms [5]. That made us curious to undertake screening of phytochemical constituents and

Corresponding Author:**Rozina Parul**

Senior Lecturer
Department of Pharmacy, Gono
Bishwabidyalay, Mirzanagor,
Savar, Dhaka, Bangladesh

pharmacological evaluation such as antioxidants, cytotoxicity, thrombolytic, antidiabetic, anti-diarrheal, and antimicrobial activity of these plants.

Materials and Methods

Collection and Identification of the Plant

The leaves of the plant *Syzygium balsameum* and *Syzygium formosum* were selected for phytochemical and pharmacological investigation. The plant, *Syzygium balsameum* formerly known as *Syzygium nervosum* & another plant *Syzygium formosum* was identified by using standard taxonomical methods at the Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. The accession numbers are respectively 47319, 47381. The plant is distributed throughout India, Myanmar, Bhutan, China and Thailand. In Bangladesh, it is found in the districts of Dhaka, Gazipur, Sylhet, and Chittagong. The leaves of the plant were collected from the forest range of Bhabanipur, Gazipur, Dhaka, and Moulvi Bazar, Sylhet Bangladesh. The cleaned leaves were dried in the shade for 7 days followed by drying in an oven at 40°C for 2 hours. The leaves were then reduced into a coarse powder with the help of a mechanical grinder. The powder of the leaves was stored in an airtight container and kept in a dark and dry place.

Extraction of the Plant Material

The maceration technique was applied for the extraction process 400g of the dried powder of the leaves of two plants were taken in a jar containing desired solvent with continuous stirring with a magnetic stirrer for seven days and changed the solvents every 48 hours. The powder was first defatted with n-hexane for seven days. The defatted powder was then successively extracted with ethyl- acetate and methanol as before, each for 7 days. The solvents were removed using a rotary evaporator at 40°C. The extracts thus obtained were separately collected. Extracts are denoted as SBH, SBEa, SBM for n-hexane, ethyl acetate and methanolic extract of *Syzygium balsameum* & SFH, SFEa, SFM for n-hexane, ethyl acetate and methanolic extract of *Syzygium formosum*.

Phytochemical Screening

Preliminary phytochemical screenings of extracts *Syzygium balsameum* and *Syzygium formosum* were carried out using standard procedures as described by Trease and Evans 1989 [6]. The freshly prepared crude extracts were qualitatively tested for the identification of chemical constituents, such as alkaloids, flavonoids, steroids, glycosides, saponins, terpenoids, carbohydrates, gums, and tannin.

Antioxidant Study

DPPH Free Radical Scavenging Assay

DPPH is a stable free radical that acts as an electron acceptor (oxidizing agent) and causes oxidation of other substances. On the other hand, antioxidants act as electron donors (reducing agent). Antioxidants neutralize DPPH by being themselves oxidized. DPPH is found as dark-colored crystalline powder and forms a deep violet color in solution. The scavenging of DPPH free radical is indicated by a deep violet color being turned pale yellow or colorless [7]. The absorbance of crude extracts/standard was measured at 517nm with a UV-Visible spectrophotometer (Shimadzu) and their DPPH free radical inhibitory potency was calculated using the following equation.

$$\% \text{ inhibition} = (C - T) / C \times 100$$

Where, C=Absorbance of control

T= Absorbance of crude extracts/standard.

Total Phenolic Content

The total phenol content in extracts was determined using Folin-Ciocalteu reagent (FCR) based on colorimetric reaction by using Harbourn *et al.*, 2009 method [8]. Gallic Acid was used as standard to produce a calibration curve. The phenol content in extracts was expressed as mg of gallic acid equivalents (GAE) /g of extract.

Total flavonoid Content

The total flavonoid content in plant extracts was determined using the colorimetric method involving aluminum chloride [9]. Quercetin was used as a reference standard and flavonoid contents of extracts were calculated as mg of QE/g of extract.

Reducing Power Capacity Assessment

The reducing power of different extracts was evaluated by the method of Oyaizu [10]. The reducing power capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

The presence of reductants such as antioxidant substances in the samples, causes the reduction of the Fe³⁺ ferricyanide complex to the ferrous form of donating an electron. The amount of Fe³⁺ complex can then be monitored by measuring the formation of Perl's Prussian blue at 700nm.

Total Antioxidant Capacity

The total antioxidant activity of the extract can be evaluated by the Phosphomolybdenum method according to the procedure of Prieto *et al.* 1999 [11]. The assay is based on the reduction of Mo (VI)-Mo (V) by the extract and subsequent formation of green PO₄/Mo (V) complex at acidic P^H.

Brine Shrimp Lethality Bioassay

Cytotoxicity of the selected extracts was determined by Brine Shrimp lethality bioassay described by Meyer *et al.* 1982 [12]. By this method natural product extracts, fractions as well as pure compounds can be tested for their bioactivity. The method utilizes in vivo lethality in a simple zoological organism (Brine nauplii) as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products.

The effectiveness or concentration-mortality relationship of plant products is usually expressed as a median lethal concentration (LC₅₀).

This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure time and is determined by the linear regression method from plotting % mortality against the corresponding log of concentration.

Thrombolytic Activity

Thrombolytic activity of all extractives was evaluated by the method developed by Dagainwala [13] using streptokinase (SK) as the standard substance. The dry extract (100mg) from each plant was suspended in 10ml of distilled water and kept overnight. Then the soluble supernatant was decanted and filtered through a 0.22-micron syringe filter.

For clot lysis, venous blood drawn from healthy volunteers was distributed in different pre-weighed sterile microcentrifuge tubes (1ml/tube) and incubated at 37°C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot and each tube having clot

was again weighed to determine the clot weight. For each microcentrifuge tube containing pre-weighted clot, 100µl aqueous solutions of different partitions and crude extract were added separately. Then, 100µl streptokinase (SK) and 100mL of distilled water were separately added to the control tube as positive and negative controls respectively. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, the release of fluid was removed and tubes were again weighed to observe the difference in weight after clot disruption. Differences obtained in weight taken before and after clot lysis were expressed as the percentage of clot lysis as showed below:

$$\% \text{ of clot lysis} = (\text{wt. of the released clot} / \text{clot wt.}) \times 100$$

Antimicrobial Screening

The antibacterial spectrum of the crude extracts is determined by observing the growth response by using the disc diffusion method [14]. The antimicrobial potential of the test agents is measured by their activity to prevent the growth of microorganisms surrounding the discs which give a clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeters with a transparent scale.

Anti-diabetic Test

Oral Glucose Tolerance Test (OGTT)

Overnight fasted normal rats weighing 150-180gm of either sex were divided into 8 groups of six animals each. Group-I served as a control group (treated with water), Group-II served as the standard group (glibenclamide 10mg/kg), Group- (III-VIII) served as n-hexane, ethyl acetate and methanol extract of *Syzygium balsameum* and *Syzygium formosum* 250mg/kg and 500mg/kg respectively. Thirty minutes after administration, glucose solution was administered orally in every group. A blood sample was drawn from the tail vein and the blood glucose level was measured by glucometer at 0, 60, and 120 minutes of treatment with glucose [15].

Antidiarrheal Test

Castor Oil-induced Diarrhea in Mice

The method was described by Jebunnessa *et al.* 2009, method was used with slight modification to evaluate the anti-diarrheal activity of the plant extracts [16].

Mice of either sex fasted for 12 h were allocated to four groups of six animals each. Group I (received DW at 10 ml/kg p.o.) served as the control group, Group II (received loperamide 10 mg/kg, p.o.) served as the standard Group- (III-VIII) served as n-hexane, ethyl acetate, and methanol extract of *S. balsameum* and *S. formosum* 250mg/kg and 500mg/kg b.w. p.o., respectively. One hour after administration, mice were fed castor oil orally at a dose of 0.5mL per mouse to induce diarrhea. The total number of both dry and wet feces excreted by the animals was counted every hour for 4 hours. The activity of each group was expressed as a percentage inhibition of defecation and percent inhibition of diarrhea [17].

Magnesium Sulfate-induced Diarrhea in Mice

Mice fasted for 12 hours and were divided into six groups of 6 mice each. Group I (received DW at a dose of 10 ml/kg p.o.) served as the control group, Group II (received loperamide 10 mg/kg, p.o.) served as standard, group- (III -VIII) served as n-hexane, ethyl acetate and methanol extract of *Syzygium balsameum* and *Syzygium formosum* 250mg/kg and 500mg/kg b.w. respectively. All treatments were given orally. After 1 hour, each mouse received MgSO₄ (2 g/kg b.w) by the oral route to induce diarrhea. The animals were placed individually in cages over a white filter paper [17]. The activity of each group was expressed as inhibition (%) of defecation and inhibition (%) of diarrhea.

Results and Discussion

Preliminary phytochemical analysis

Preliminary phytochemical screening of the different extracts of *Syzygium balsameum* & *Syzygium formosum* revealed the presence of various bioactive components of which carbohydrates, glycosides, flavonoids, alkaloids, tannins. (Table 1).

Table 1: Results of preliminary phytochemical analysis

Phytochemical Investigated	SBH	SBEa	SBM	SFH	SFEa	SFM
Glycoside	(+)	(+)	(+)	(-)	(-)	(+)
Carbohydrates	(+)	(+)	(+)	(+)	(+)	(+)
Saponins	(-)	(-)	(+)	(-)	(+)	(+)
Alkaloids	(-)	(-)	(+)	(+)	(+)	(+)
Steroids	(+)	(+)	(-)	(-)	(-)	(-)
Tannins	(-)	(+)	(+)	(+)	(+)	(+)

*symbol (+) indicates presence and symbol (-) indicates absence

DPPH Free Radical Scavenging Assay

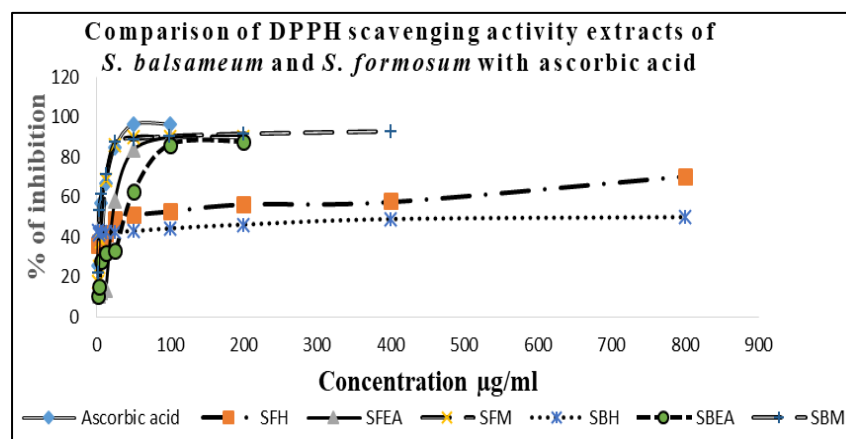
The DPPH free radical scavenging assay of the n-hexane, ethyl acetate, and methanol extracts of *S. balsameum* and *S. formosum* (leaves) were assessed as IC₅₀ value. In the present study, methanol extracts of plants showed scavenging of DPPH free radicals in a way similar to that of the standard antioxidant ascorbic acid.

However, the exhibited antioxidant potency (IC₅₀ values of hexane, ethyl acetate, and methanol extracts of *S. balsameum* are 676.146, 133.831, 2.814, *S. formosum* are 183.235, 38.795, 13.535µg/ml respectively and ascorbic acid 2.857. The results showed that methanol extracts of plants close as to the antioxidant effect of ascorbic acid (table 2 and fig 1), indicates good antioxidant property.

Table 2: IC₅₀ values of DPPH scavenging activity of the ascorbic acid and extracts of *S. balsameum* and *S. formosum*.

Sample / Standard	IC ₅₀ (µg/ml)
SBH	676.146
SBEa	133.831
SBM	2.814
SFH	183.235
SFEa	38.795
SFM	13.535
Ascorbic acid	2.857

*Results are express as mean ±SD

**Fig 1:** DPPH free radical scavenging activity of ascorbic acid and extracts of *S. balsameum* and *S. formosum*.**Total Phenolic Content**

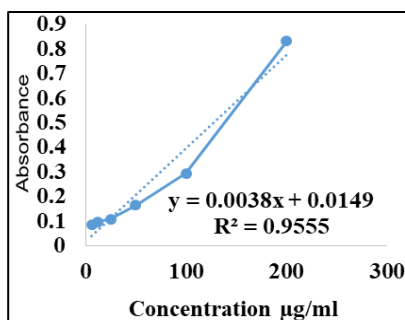
The total phenolic contents of the n-hexane, ethyl acetate, and methanol extracts of *Syzygium balsameum* and *Syzygium formosum* (leaves) were calculated using the standard curve of gallic acid ($y=0.0038x+0.0149$, $R^2 = 0.9555$). The study is

exhibited that all the extracts have phenolic content. But methanol extracts show a higher amount of total phenolic content (*S. balsameum* 24.74 ± 0.055 & *S. formosum* 24.34 ± 0.020) table 3 and fig 2.

Table 3: The total phenolic content

Extract	Total phenolic content (mg/g, gallic acid equivalents)
SBH	6.610 ± 0.089
SBEa	6.347 ± 0.013
SBM	24.74 ± 0.055
SFH	7.873 ± 0.021
SFEa	19.215 ± 0.082
SFM	24.34 ± 0.020

*Results are express as mean ±SD

**Fig 2:** Standard curve of gallic acid**Total Flavonoid Content**

The total flavonoid contents of the n-hexane, ethyl acetate, and methanol extracts of *Syzygium balsameum* and *Syzygium formosum* (leaves) were calculated using the standard curve of quercetin ($y = 0.007x - 0.00183$; $R^2 = 0.9962$) and are expressed as quercetin equivalents (QE) per gram of the plant extracts.

Total flavonoids content study shows that the *S. balsameum* methanol extract (49.75 ± 0.053) and *S. formosum* ethyl acetate extract (42.311 ± 0.002) greater total flavonoids content compared to other extracts (table 4 and fig 3).

Table 4: Total flavonoids content

Extract	Total flavonoids content (mg/g, quercetin equivalents)
SBH	18.08 ± 0.015
SBEa	19.42 ± 0.009
SBM	49.75 ± 0.053
SFH	14.53 ± 0.014
SFEa	42.311 ± 0.002
SFM	15.08 ± 0.002

Results are express as mean±SD

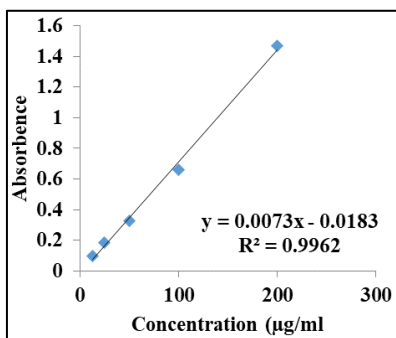


Fig 3: Standard curve of gallic acid.

In this study, all the extracts of both species showed mild antioxidant capacity, total antioxidant capacity given in table 5, and fig 4.

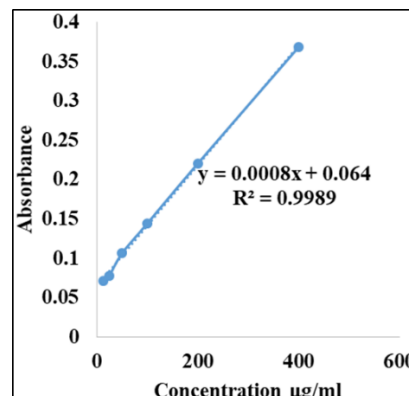


Fig 4: Calibration curve of ascorbic acid

Total antioxidant capacity

The total antioxidant capacity of the n-hexane, ethyl acetate, and methanol extracts of *Syzygium balsameum* and *Syzygium formosum* (leaves) were assessed using the standard curve of ascorbic acid ($y = 0.0008x + 0.064$, $R^2 = 0.9989$) and are expressed as ascorbic acid equivalents per gram of the plant extracts.

Table 5: Total antioxidant capacity.

Extract	Total Antioxidant Capacity (mg/gm, Ascorbic Acid Equivalent)
SBH	12.26± 0.009
SBEa	8.85± 0.001
SBM	10.95± 0.001
SFH	6.26± 0.000
SFEa	9.41± 0.000
SFM	12.78± 0.002

*Results are express as mean±SD

Cytotoxicity test by brine shrimp lethality bioassay

The cytotoxic activity of all the extract was determined by Brine Shrimp lethality bioassay. After 24 hours, the test tubes were inspected and the number of surviving nauplii in each tube was counted. From this data, the percentage of lethality

was calculated for each concentration. Cytotoxicity study of the extracts exhibited insignificant cytotoxicity compared to the standard vincristine sulfate. % mortality and LC_{50} values of the extracts is given below in fig 5 & 6.

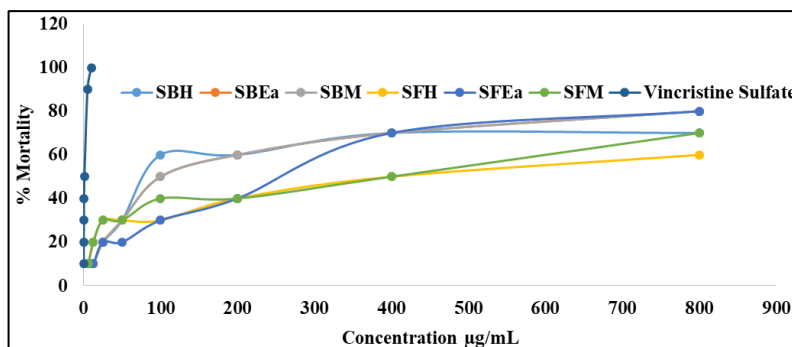


Fig 6: LC_{50} value of different extracts of the plants and vincristine sulfate.

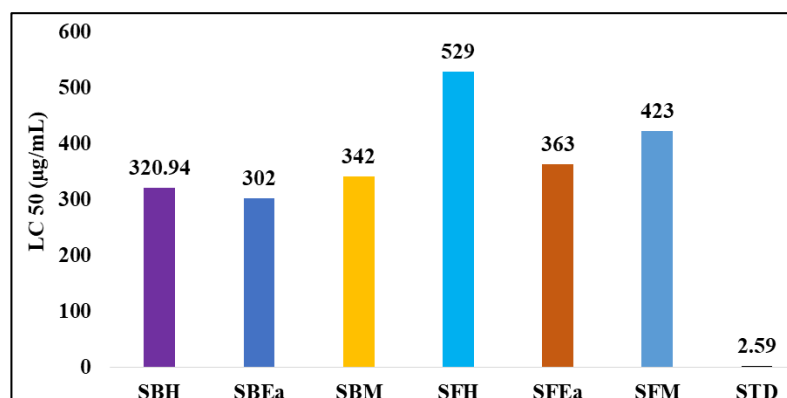


Fig 6: LC_{50} value of different extracts of the plants and vincristine sulfate.

Thrombolytic Activity

Thrombolytic Activity of the n-hexane, ethyl acetate, and methanol extracts of *Syzygium balsameum* and *Syzygium formosum* (leaves) was assessed for thrombolytic activity and the results are presented in table 7. The addition of 100 μ L of streptokinase, a positive control, to the clots and subsequent incubation for 90 minutes at 37°C, showed 65% lysis of the

clot. On the other hand, distilled water was treated as a negative control which exhibited a negligible percentage of lysis of clots. In this study crude hexane, ethyl acetate, and methanol soluble fractions of *Syzygium balsameum* and *Syzygium formosum* (leaves) exhibited mild thrombolytic activity compared with standard streptokinase in table 7 and fig 7.

Table 7: Thrombolytic activity of various extracts of *S. balsameum* and *S. formosum* (leaves)

Sample	Wt. of empty vial (W1g)	Wt. of clots containing vial before clot disruption (W2g)	Wt. of clots containing vial after clot disruption (W3g)	Wt. of clots before disruption clot (W4=W2-W1g)	Wt. of clots after disruption clot (W5=W3-W1g)	% of clot lysis W4-5/W4*100%
(blank)	4.7402	5.6650	5.6278	0.9248	0.8876	4.02%
Streptokinase	4.6	5.05	4.79	0.4	0.14	65%
SBH	4.8041	5.5922	5.4200	0.7881	0.6159	21.85%
SBEa	4.7898	5.6973	5.6824	0.9075	0.8926	29.72%
SBM	4.83	5.63	5.62	0.8	-0.01	20.60%
SFH	5.3208	6.1696	6.1262	0.8488	0.8054	25.92%
SFEa	4.77	5.7	5.27	0.93	0.5	31.36%
SFM	4.7337	5.5900	5.4183	0.8563	0.6846	20.05%

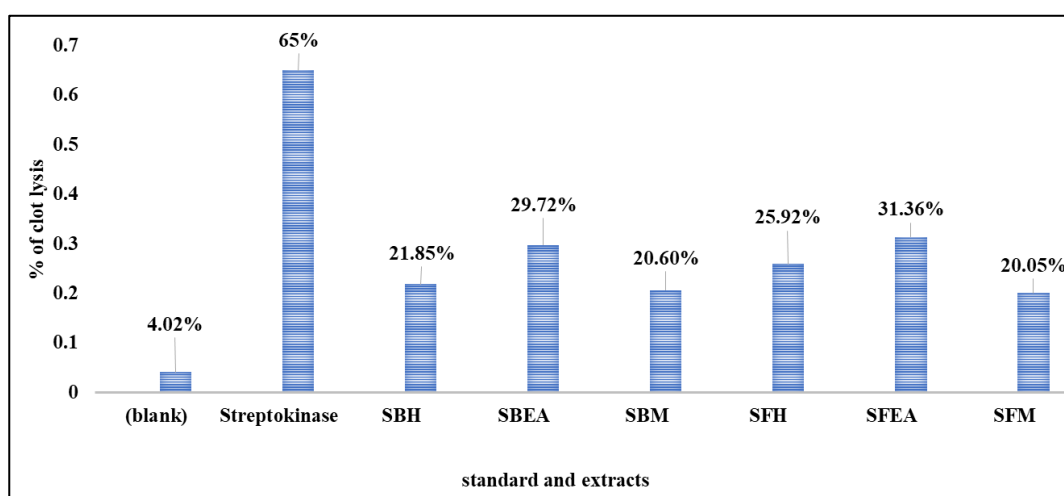


Fig 7: Evaluation of thrombolytic effect

Antimicrobial Activity

The result of antimicrobial screening of different extracts of *S. balsameum* and *S. formosum* has been presented in table 8. All the extracts of *S. balsameum* & *S. formosum* leaves showed mild antimicrobial activity against the gram-negative bacteria. Only SBEa showed no zone of inhibition against *Klebsiella michagenensis*. *Syzygium balsameum* & *Syzygium*

formosum leaf extracts showed good antimicrobial activity against *Bacillus Cereus* and *Bacillus subtilis* compared to standard except SBM extract. The SBM extract and other extracts showed moderate activity against gram-negative and other gram-positive bacteria. We used several antibiotics due to the avoid the resistance of microorganisms.

Table 8: Antimicrobial activity of the extracts of *S. balsameum*, *S. formosum*, and standard.

Test Organisms	Inhibition Zone Diameter (mm)						
	Std.	SBH	SBEa	SBM	SFH	SFEa	SFM
<i>Escherichia coli</i> (EPEC)	LEV (5) - 33	8	8	9	12	7	7
<i>Escherichia coli</i> (ETEC)	LEV (5) - 33	8	9	10	7	9	10
<i>Escherichia coli</i> (ATCC)	FEP (30) - 40	8	8	5	10	5	12
<i>Salmonella paratyphi</i>	LEV (5) - 26	10	8	7	8	10	13
<i>Salmonella typhi</i>	GN (10) - 30	12	8	8	11	9	9
<i>Klebsiella michagenensis</i>	CRO (30) - 28	6	nzd	8	7	6	6
<i>Klebsiella spp</i>	GN (10) - 23	7	7	8	9	7	7
<i>Klebsiella oxytoca</i>	GN (10) - 27	8	6	9	6	6	9
<i>Klebsiella pneumoniae</i>	LEV (5) - 26	12	10	7	7	9	8
<i>Proteus vulgaris</i>	LEV (5) - 25	7	8	7	7	9	11
<i>Bacillus Cereus</i>	CRO (30) -10	9	10	9	12	10	8
<i>Bacillus subtilis</i>	VA (30) - 21	13	9	6	10	13	15
<i>Staphylococcus aureus</i>	CRO (30) - 22	7	8	9	10	11	10
<i>Enterococcus faecalis</i>	LEV (5) - 43	7	9	10	8	8	7

* LEV= Levofloxacin 5 μ g, FEP = Cefepime 30 μ g, GN = Gentamycin 10 μ g, CRO=Ceftriaxone 30 μ g, nzd= No zone detected

Anti-diabetic activity

Oral glucose tolerance test (OGTT): OGTT is a primary screening test for the hypoglycemic effect. The results of the OGTT test of *Syzygium balsameum* and *Syzygium formosum* at 250 & 500mg/kg of SFH & SFEa showed insignificant

activity. Significant activity in SBM & SFM and SBH & SBEa has no hypoglycemic effect as compared to the vehicle-treated control group and standard. The results are given below in the table 9 & 10.

Table 9: OGTT for hexane, ethyl acetate, and methanolic extract of *Syzygium balsameum*

Group	0 min (mmol/L)	60 min (mmol/L)	120 min (mmol/L)
Control	6.51±0.46	20.30±1.62	14.56±1.52
Std.	7.08±0.29	7.00±1.62	6.23±0.81
SBH 250	8.85±0.53	19.23±1.73	14.20±1.29
SBH 500	7.23±0.36	21.53±0.86	16.86±1.84
SBEa 250	6.38±0.57	21.05±1.41	16.08±1.62
SBEa 500	7.35±0.46	18.85±1.42	15.25±1.80
SBM 250	5.58±0.64	6.31±0.27	6.51±0.29
SBM 500	4.43±0.36	6.43±0.59	5.31±0.18

Table 10: OGTT for hexane, ethyl acetate, and methanolic extract of *Syzygium formosum*

Group	0 min (mmol/L)	60 min (mmol/L)	120 min (mmol/L)
Control	6.93±0.25	11.68±1.86	7.98±0.57
Std.	6.58±0.29	7.00±0.65	6.23±0.91
SFH 250	6.93±0.25	7.78±0.37	6.98±0.21
SFH 500	6.76±0.57	6.44±0.49	5.70±0.37
SFEa 250	5.93±0.37	9.13±0.18	8.15±0.16
SFEa 500	4.86±0.39	8.88±0.29	8.13±0.33
SFM 250	5.08±0.60	6.63±1.36	6.01±0.54
SFM 500	4.68±0.58	5.65±2.15	6.20±1.80

Castor Oil and Magnesium Sulfate-Induced Diarrhea in Mice

Antidiarrheal activity of all extracts of *Syzygium balsameum* and *Syzygium formosum* (leaves) was expressed as percent inhibition of defecation and percent inhibition of diarrhea. The results showed that there has been a statistically insignificant reduction in the incidence and severity of diarrhea with a higher dose of all the extracts of *S. balsameum*

and *S. formosum* in experimental animals. Magnesium sulfate acts as a laxative. The extract SBH, SBEa and SBM reduced the total number of feces and diarrheal episodes insignificantly at both doses in MgSO₄ induced diarrhea. The SFH, SFEa, and SFM didn't have any capacity to defend against diarrheal episodes. The standard drug loperamide showed marked antidiarrheal efficacy in both models (table 11, 12, 13 & 14).

Table 11: Castor oil-induced antidiarrheal test for SBH, SBEa, SBM at 250 & 500mg/Kg.

Group	Total feces (mean ± SEM)	% inhibitor of total feces	Diarrheal feces ± (SME)	% inhibitor of diarrhea
Control	8.00±1.59	00	1.66±0.49	00
Std.	2.00±0.44	75	0.66±0.49	60.24
SBH 250	4.50±1.52	43.75	2.00±0.77	-20.48
SBH 500	4.16±0.70	47.92	1.50±0.67	9.63
SBEa 250	7.50±1.33	19.64	4.16±0.98	34.21
SBEa 500	5.16±1.75	44.64	3.83±1.47	39.47
SBM 250	15.00±1.97	-57.89	4.20±0.735	-93.84
SBM 500	8.66±1.87	8.77	3.00±0.68	-38.46

Table 12: Mg sulfate-induced antidiarrheal test for SBH, SBEa, SBM at 250 & 500mg/Kg.

Group	Total feces (mean ± SEM)	% inhibitor of total feces	Diarrheal feces ± (SME)	% inhibitor of diarrhea
Control	9.50±1.43	00	5.16±1.19	00
Std	2.33±0.71	75.47	1.00±0.36	80.62
SBH 250	7.33±1.78	22.84	5.50±0.92	-6.58
SBH 500	6.83±2.41	28.10	2.33±1.17	54.84
SBEa 250	9.16±1.19	3.51	8.0±0.96	-54.83
SBEa 500	6.66±1.33	29.82	5.33±1.14	-3.22
SBM 250	3.00±0.83	00	3.00±0.441	-20
SBM 500	1.66±0.66	44.44	1.50±0.42	40

Table 13: Castor oil-induced antidiarrheal test for SFH, SFEa, SFM at 250 & 500mg/Kg.

Group	Total feces (mean ±SEM)	% inhibition of total feces	Diarrheal feces± (SME)	% inhibition of diarrhea
Control	9.66±2.14	00	7.50±1.58	00
Std	2.83±1.27	70.69	1.66±0.76	77.86
SFH 250	5.83±1.92	39.65	4.33±1.97	42.22
SFH 500	10.00±1.06	-3.51	5.33±1.08	28.93
SFEa 250	1.00±0.36	-525	4.50±1.05	-93.13
SFEa 500	1.50±0.42	-837.5	4.66±0.49	-100
SFM 250	9.50±1.80	-96.68	6.16±1.44	-185.18
SFM 500	8.50±1.43	-75.98	4.33±0.84	-100.46

Table 14: Mg sulfate-induced antidiarrheal test for SFH, SFEa, SFM at 250 & 500mg/Kg.

Group	Total feces (mean \pm SEM)	% inhibition of total feces	Diarrheal feces \pm (SME)	% inhibition of diarrhea
Control	7.66 \pm 1.08	00	3.16 \pm 0.83	00
Std	1.33 \pm 0.71	82.63	1.0 \pm 0.63	68.35
SFH 250	5.83 \pm 1.55		2.50 \pm 1.08	20.88
SFH 500	6.66 \pm 2.09	13.05	4.50 \pm 1.31	-0.42
SFEa 250	1.80 \pm 0.37	-200	4.20 \pm 0.48	-10.52
SFEa 500	1.60 \pm 0.50	-166.66	4.80 \pm 0.48	-26.31
SFM 250	8.33 \pm 1.11	10.71	3.83 \pm 1.51	0
SFM 500	6.00 \pm 2.12	35.69	3.83 \pm 1.74	0

Conclusion

The findings of the present study suggested methanol extracts of the leaves of *S. balsameum* & *S. formosum* show more biologically active than other non-polar solvent extracts and also suggested that the leaves of the two plant have significant medicinal value such as antioxidant, antidiabetic, antimicrobial, etc. due to the presence of several phytochemicals. Further study is required to identify the underlying mechanism of action and bioassay-guided fractionation to isolate the active constituents from these plants for the development of new lead compounds.

Acknowledgments

The authors are grateful to the Department of Pharmacy Gono Bishwbidyalay, Savar, Dhaka-1344, and Department of Pharmacy, Jahngirnagar University, Savar, Dhaka for the laboratory facility to conduct the research work.

Conflict of interest

All the authors declared that they do not have any conflicts of interest in publishing this research article.

References

1. Firenzuoli F, Gori L. Herbal medicine today; clinical and research issues. *Evid Based Complement Alternat Med* 2007;4(Suppl 1):37-40.
2. Sircar NN. Medicinal plants. *The Eastern pharmacist* 1982;29(291):49-52.
3. Hamilton AC. Medicinal plants, conservation and livelihoods. *Biodiversity and Conservation* 2004;13(8):1477-1517.
4. Ghani A. *Text Book of Pharmacognosy*. 2nd Edition Parash Publishers, Dhaka, Bangladesh 2005;197-205.
5. Nguyen TMN, Lomunova M, Vu TPD, Le BV, KimYH, Kang JS, *et al*. Anti-allergic effects of the ethanol extract of *Syzygium formosum* (Wall.) Masam leaves and its immunoregulatory mechanisms. *Journal of Ethnopharmacology* 2018;30(211):171-179.
6. Evans WC. *Trease and Evan's Textbook of Pharmacognosy*, 13th Edition, Cambridge University Press, London 1989, 546.
7. Braca A, Tomashi ND, Bari LD, Pizza C. Antioxidant principles from *Bauhinia terapotensis*. *Journal of Natural Products* 2001;64:892-895.
8. Harbourne N, Marete E, Jacquier JC, Riordan DO. Effect of drying methods on the phenolic constituents of meadowsweet (*Filipendula ulmaria*) and willow (*Salix alba*). *LWT - Food Science and Technology* 2009;42(9):1468-1473.
9. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in Propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis* 2002;10(3):178-182.
10. Oyaizu M. Studies on Products of Browning Reactions: Antioxidative Activities of Product of Browning Reaction Prepared from Glucosamine. *Japan Journal of Nutrition* 1986;44:307-315.
11. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific Application to the determination of vitamin E. *Analytical Biochemistry* 1999;169(2):337-341.
12. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL, *et al*. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica* 1982;45(5):31-34.
13. Dagainawala HF, Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, *et al*. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. *Thrombosis Journal* 2006;4:14.
14. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standard single disk method. *American Journal of Clinical Pathology* 1966;45:493-496.
15. Joy KL, Kuttan R. Anti-diabetic activity of *Picrorrhiza kurroa* extract. *Journal of Ethnopharmacology* 1999;67(2):143-148.
16. Jebunnessa, Uddin SB, Zaman MM, Akter R, Ahmed NU. Antidiarrheal activity of ethanolic bark extract of *Mitragyna diversifolia*. *Bangladesh Journal of Pharmacology* 2009;4(2):144-146.
17. Imam MZ, Sultana S, Akter S. Antinociceptive, antidiarrhoeal and neuropharmacological activities of *Barringtonia acutangula*. *Pharmaceutical Biology* 2012;50(9):1078-1084.