

Phytochemical Analysis and Repellent Activity of *Annona Muricata* and *Syzygium Samarangense* Leaves Against *Sitophilus Oryzae*

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ABSTRACT

The present study is conducted to control the stored pest grain - *Sitophilus oryzae* using plant leaves, such as *Annona muricata* and *Syzygium samarangense*. Extraction of selected leaves were done using organic solvents such as Methanol and petroleum ether. Qualitative phytochemical analysis of selected plant leaves revealed the presence of secondary metabolites such as Alkaloids, Flavonoids, Tannins, Saponins, Glycerides, Steroids, Terpenoids, Phenols, Quinone and Phlobotannine, which have repellent activities against *Sitophilus oryzae*. Repellency test were also done using Methanol and petroleum ether leaf extract *Annona muricata* and *Syzygium samarangense*. No repellent activity were observed in Petroleum ether leaf extract of *Annona muricata* in all concentration. Methanol leaf extract of *Annona muricata* showed highest repellency of 61.33% at 6.4% concentration in 24 hrs. Petroleum ether leaf extract of *Syzygium samarangense* showed highest repellency of 55.00% at 6.4% concentration in 24 hrs, where on methanol leaf extract of *Syzygium samarangense* showed highest repellency of 60.00% at 6.4%. These leaf extracts can be used as an alternative biopesticides instead of chemical pesticides, which would be safer for storing rice grains.

Keywords: *Syzygium samarangense*, *Annona muricata*, *Sitophilus oryzae*, Botanical pesticides, Phytochemical analysis, Repellent activity.

1. INTRODUCTION

Botanical insecticides are applied to control insect from use of essential oils, flavonoids, alkaloids, glycosides, esters, and fatty acids having anti-insect effect use to remove insect [1]. Each plant contains of numerous significant components that can be used in medical field, and can be involved in the development of diverse kind of drugs [2]. Several products of plants have been tried with a good degree of success as protectants against a number of stored grain insect pests [3]. The study showed the presence of secondary metabolites in *Annona muricata* and *Syzygium samarangense* and the main aim is to estimate the repellent properties of crude extracts of above plants against Stored pest *Sitophilus oryzae*.

2. MATERIALS AND METHODS

2.1 PLANT MATERIAL COLLECTION AND PREPARATION OF EXTRACTS

The new leaves of *Annona muricata*, *Syzygium samarangense* were collected from surroundings of Thikkodi Gramapanchayat. The leaves were clean, dried in sunlight, ground into powder, and kept at 20°C for further studies. The leaf powder of 30 g was extracted in 100 ml methanol or petroleum ether in Soxhlet extraction apparatus at 60°C for 48 hrs. The crude extract was clean, evaporated under reduced pressure at 70°C in a rotary vacuum evaporater, and then dried in 80°C lyophilizer, the lyophilized powder was stored at 4°C for 48 hrs, [4]. Extracts of different samples were prepared rendering to methods developed by Wagner H *et al.*, Khandelwal KR. and Mukherjee PK.

2.2 RICE WEEVIL REARING

Adult rice weevils (*S. oryzae*) were obtained from naturally infested milled rice. The insect was reared in a plastic container (3.7 cm height, 7.5 cm diameter) under laboratory conditions at 28 ± 2°C, 65-70% relative humidity, 12-hr light, 12hr dark and covered with a fine mesh cloth for ventilation. This jasmine rice (*Oryzae sativa*) var. KDML 105 was used in all experiments. The container was returned to the rearing and the culture was continued for a month to allow new adults emerged. The insects of 7-10 days old were used for the experiments.

2.3 PHYTOCHEMICAL ANALYSIS

Qualitative analysis

Preliminary phytochemical analysis [5] were carried out to test the presence of Tannins, Flavonoids, Terpenoids, Alkaloids, Steroids, Saponins, Quinones, Glycosides, Phenols and Phlobotannins.

1) DETECTION OF ALKALOIDS

The extracts were dissolved in 1 ml of dilute sulphuric acid and filtered using Whatman No.1 filter paper and the filtrate treated with Mayer's reagent. The appearance of cream colour indicates the presence of alkaloids.

2) DETECTION OF FLAVONOIDS

The extracts were mixed with 1-2 ml of ammonia and add 1-2 ml of sulphuric acid. Presence of yellow colour indicate the presence of Flavanoids.

3) DETECTION OF TANNINS

The extracts were dissolved in 10 ml of distilled water and allowed to settle and filtered. To the filtrate 1- 2 ml of 5% ferric chloride was added. The appearance of deep green colour indicates the presence of tannin. Another portion of the filtrate was treated with 1-2 ml of iodine solution and a faint bluish colour confirmed the presence of tannin.

4) DETECTION OF SAPONINS

About 1 ml of test extract was dissolved in 20 ml of distilled water and shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

5) DETECTION OF GLYCOSIDES

The extracts were dissolved in 10 ml of diluted water under boiling conditions. This was filtered and 2 ml of the filtrates hydrolysed with a few drops of concentrated HCL and the solution was rendered alkaline with 1-2 drops of ammonia solution. Five drops of this solution were added to 2 ml of Benedict's qualitative reagent and boiled. A reddish-brown precipitate showed the presence of glycosides.

6) DETECTION OF STEROIDS

The extracts were dissolved in 2 ml of chloroform. To this 1 ml of concentrated sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interface indicates the presence of steroids.

7) DETECTION OF TERPENOIDS

The extracts were dissolved in 1ml of chlorofoam and 1 ml of acetic anhydride. To this solution 2 drops of concentrated sulphuric acid were added. A pink colour which changes to bluish green on standing indicated the presence of Terpenoids.

8) DETECTION OF PHENOLS

The extracts were dissolved in 10 ml of water and ferric chloride solution (5%) or gelatin solution (1%) or lead acetate solution (10%). The appearance of deep blue colour with ferric chloride or precipitation with other reagents indicates the presence of total phenols.

9) DETECTION OF QUINONE

About 2 ml of test extract was added to 3 ml of hydrochloric acid. The appearance of a yellow precipitate indicates the presence of Quinone.

10) DETECTION OF PHLOBOTANIN

About 2 ml extract was added to 2 ml 1% HCl. Formation of red precipitate indicates the presence of phlobotanin.

2.4 REPELLENT TEST OF PLANT LEAF EXTRACTS

The effects of crude extracts on repellence were conducted by a cone bioassay. The setting was as in. Twenty grams of milled grains were thoroughly mixed with 0.8, 1.6, 3.2 and 6.4% extracts. The mixed grains were put in a small plastic bottle. The small plastic bottle placed in a large bottle. The adult weevils were introduced into the middle of the mixed grains put in small plastic bottle. The weevils those escaped out from the cone were counted after 15, 30, and 60 minutes, 1, 12 and 24 hrs. 1% methanol and petroleum ether were used as control. The bottle contained 20 gm milled rice grains which were mixed with various concentrations of extracts. The weevils were introduced into the middle of bottle. The escaped *sitophilus oryzae* would fall on plate.

2.5 STATISTICAL ANALYSIS

All experiments were performed in triplicate and data are the Mean \pm S.E. Data were subjected to one-way analysis of variance (ANOVA). The means were separated using the Duncan multiple rang tests when ANOVA was significant ($P < 0.05$).

3. RESULT AND DISCUSSION

3.1 PHYTOCHEMICAL ANALYSIS

The results of Qualitative phytochemical analysis of Petroleum ether and Methanol leaf extract of *Annona muricata* and *Syzygium samarangense* were shown in (Table 1 and 2).

Petroleum ether leaf extract of *Annona muricata* showed the presence of Tannins and Steroids. Whereas, Alkaloids, Flavonoids, Saponins, Glycerides, Terpenoids, Phenols, Quinone and phlobotanin were absent. In Methanol leaf extract of *Annona muricata* showed the presence of Alkaloids, Flavonoids, Tannins, Saponins, Glycerides, Steroids, Terpenoids and Phenols. Whereas, Quinone and Phlobotannine were absent. The petroleum ether leaf extract of *Syzygium samarangense* showed the presence of Tannins Steroids, Flavonoids, Terpenoids and Phenols. Whereas Quinone, and Phlobotannine Alkaloids, Saponins, Glycerides were absent. In methanolic leaf extracts of *Syzygium samarangense* showed the presence of Flavonoids, Tannins, Saponins, Glycerides, Steroids, Terpenoids and Phenols were present. Whereas, Alkaloids, Steroids and Phlobotannins were absent.

Phytochemical analysis of the leaf extract of the *Annona muricata* plant shows the presence of secondary metabolites like tannins, steroids, cardiac glycosides, etc [7]. Phytochemical screening of all solvent extracts of *Annona muricata* leaf revealed the presence of alkaloids, carbohydrates, saponins, proteins, amino acids, phytosterols, coumarins, fats and oils, phenolic and flavonoids [8]. The phytochemical analysis of *Annona muricata* dried and powdered leaves revealed the quantitative percentage of ascorbic acid, beta-carotene, flavonoids, alkaloids, tannins, saponins and reducing sugars [9]. The leaves of *Syzygium samarangense* contained many compounds, which are flavonoids, Phenolic compounds, tannins, terpenoids, and sterols. Other metabolite groups, such as volatile Oils, Sysamarins F-

G [10]. Methanolic root extract of *Syzygium samarangense* showed the presence of tannins, flavonoids, terpenoids, and phenols [11]. The leaves of *Syzygium samarangense* contained many compounds, which are flavonoids, phenolic compounds, tannins, terpenoids, and sterols [12].

3.2 REPELLENT ACTIVITY

The repellent effects of Petroleum ether leaf extract of *Annona muricata* showed no repellency effects, in any concentration.

The repellent effects of methanol leaf extract of *Annona muricata* approximately increased from 11.66% to 41.33% at 0.8%. At 1.6% the repellent effects of methanol leaf extract of *Annona muricata*, approximately increased from 21.33% to 50.66%. At 3.2% the repellent effects methanol leaf extract of *Annona muricata* leaf methanol extract approximately increased from 31.61% to 56.00%. At 6.4% the repellent effects of methanol leaf extract of *Annona muricata* approximately increased from 41.33% to 61.33%. All treatments were compare with control (Table-3). The repellent effects of Petroleum ether leaf extract of *Syzygium samarangense* extract approximately increased from 21.33% to 37.67% at 0.8%. At 1.6% the repellent effects of approximately Petroleum ether leaf extract of *Syzygium samarangense* increased from 25.33% to 44.01%. At 3.2% the repellent effects of Petroleum ether leaf extract of *Syzygium samarangense* extracts approximately increased from 30.33% to 50.66%. At 6.4% the repellent effects of Petroleum ether leaf extract of *Syzygium samarangense* approximately increased from 35.33% to 55.00%. All treatments were compare with control. (Table-4).

The repellent effects of methanol leaf extract of *Syzygium samarangense* approximately increased from 12.66% to 34.33% at 0.8%. At 1.6% the repellent effects of methanol leaf extract of *Syzygium samarangense* approximately increased from 21.10% to 42.01%. At 3.2% the repellent effects of methanol leaf extract of *Syzygium samarangense* approximately increased from 31.33% to 51.66%. While the methanol leaf extract of *Syzygium samarangense* shows highest repellency 60.00% at the concentration 6.4% and 24 hrs. Methanolic leaf extract of *Annona muricata* shows highest repellency of 61.33% at the concentration of 6.4% in 24 hrs. Petroleum ether leaf extract of *Syzygium samarangense* shows lowest repellency 55% at the concentration of 6.4% in 24 hrs (Table-5).

Jayakumar, Arivoli, Raveen, and Tennyson, (2017) reported that repellent activity of camphor, citronella, eucalyptus, lemon and wintergreen oil essential oils against stored product pests; the adult rice weevil *Sitophilus. oryzae* Linnaeus 1763 (Coleoptera: Curculionidae), due to nor attractant activity [13]. *Annona muricata* and *Syzygium samarangense* extract exhibited high repellency against rice weevils, which was similar to the neem acetone extract [14] and to the extract of *Urca dioica* L and *Taraxacum officinale* [15]. There were some evidences supported that the compounds present in *Annona muricata* and *Syzygium samarangense* exerted a toxic effect by troublemaking normal respiratory activity of the weevils resulting in asphyxiation and subsequent death [16]. Some plants contained irritant and foul-smelling chemicals to which strongly repelled stored product insect pest [17], [18]. These supports the repellent activities of our studied plants to the stored grain pest.

Table 1. Qualitative Phytochemical Screening of Petroleum Ether and Methanol leaf Extract of *Annona Muricata*

Sl.no	Compounds	Petroleum ether	Methanol
1	Alkaloids	-	+
2	Flavanoids	-	+
3	Tannins	+	+
4	Saponins	-	+
5	Glycerides	-	+
6	Steroids	+	+
7	Terpenoids	-	+
8	Phenols	-	+
9	Quinone	-	-
10	Phlobotannine	-	-

Table 2. Qualitative phytochemical screening of Petroleum ether and Methanol leaf Extract of *Syzygium samarangense*

Sl.NO	Compounds	Petroleum Ether	Methanol
1	Alkaloids	-	-
2	Flavanoids	+	+
3	Tannins	+	+
4	Saponins	-	+
5	Glycerides	-	+
6	Steroids	+	+
7	Terpenoids	+	+
8	Phenols	+	+
9	Quinone	-	-
10	Phlobotannins	-	-

Table 3. Comparison of Repellent activities of Methanol Leaf extract of *Annona Muricata* on Adult Rice Weevils in 20 grams rice grains

Concentration %	% Repellent activity (Mean ± SE)				
	15 min	30 min	1 hr	12 hr	24 hr
0.8	11.66±0.33 ^c	14.66±0.88 ^a	22.66±0.33 ^a	32.66±0.66 ^b	41.33±0.66 ^e
1.6	21.33±0.88 ^b	24.33±0.33 ^a	28.01±0.33 ^c	38.33±0.33 ^d	50.66±0.33 ^e
3.2	31.61±0.33 ^b	35.33±0.81 ^b	44.33±0.66 ^a	47.66±0.88 ^b	56.00±0.57 ^e
6.4	41.33±0.88 ^a	43.01±1.73 ^b	48.33±0.33 ^e	54.66±0.33 ^d	61.33±0.66 ^a
Control	0.00±0.00	0.00±0.00	0.00±0.00\	0.00±0.00	0.00±0.00

a-f means within a column followed by different letters are significantly, P<0.05, Duncan multiple rank test (DMRT).

Table-4. Comparison of Repellent activities of the Petroleum ether Leaf extracts of *Syzygium samarangense* on adult rice weevil in 20 grams rice grains

Concentration %	% Repellent activity (Mean \pm SE)				
	15 min	30 min	1 hr	12 hr	24 hr
0.8	21.33 \pm 0.88 ^a	24.33 \pm 0.33 ^c	26.66 \pm 0.33 ^d	31.33 \pm 0.66 ^b	37.67 \pm 0.33 ^c
1.6	25.33 \pm 0.88 ^b	28.66 \pm 0.67 ^d	31.66 \pm 0.88 ^c	34.67 \pm 0.33 ^b	44.01 \pm 0.57 ^a
3.2	30.33 \pm 0.33 ^e	34.00 \pm 0.57 ^d	37.66 \pm 0.33 ^a	41.33 \pm 0.88 ^c	50.66 \pm 0.33 ^a
6.4	35.33 \pm 0.57 ^a	39.33 \pm 0.33 ^c	43.67 \pm 0.66 ^b	48.00 \pm 0.57 ^d	55.00 \pm 0.88 ^c
Control	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00

a-f means within a column followed by different letters are significantly, P<0.05, Duncan multiple rank test (DMRT).

Table-5. Comparison of repellent activities of the Methanol leaf extracts of *Syzygium samarangense* on adult rice weevil in 20 grams rice grains

Concentration %	% Repellent activity (Mean \pm SE)				
	15 min	30 min	1 hr	12 hr	24 hr
0.8	12.66 \pm 0.33 ^b	21.33 \pm 0.88 ^c	24.33 \pm 0.88 ^c	31.33 \pm 0.88 ^a	34.33 \pm 0.66 ^b
1.6	21.10 \pm 0.57 ^b	27.33 \pm 0.33 ^a	32.33 \pm 0.33 ^a	38.33 \pm 0.33 ³	42.01 \pm 0.57 ^b
3.2	31.33 \pm 0.66 ^b	36.66 \pm 0.88 ^c	42.01 \pm 0.57 ^d	44.66 \pm 0.88 ^e	51.66 \pm 0.33 ^a
6.4	36.66 \pm 0.88 ^e	43.33 \pm 1.20 ^a	51.00 \pm 1.20 ^a	53.01 \pm 0.57 ^b	60.00 \pm 0.66 ^a
Control	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00

a-f means within a column followed by different letters are significantly, P<0.05, Duncan multiple rank test (DMRT).

4. CONCLUSION

Medicinal plants were the powerful source of health due to its active compounds that is responsible for its numerous pharmacological activities. The result of this study revealed that application of *Annona muricata* and *Syzygium samarangense* leaf extracts improved adult repellency. Extraction from the leaves of *Annona muricata* and *syzygium samarangense* are composed of diverse phytochemicals. The present study conclusively revealed that that *Annona muricata* is a good source of several phytochemicals like alkaloids, flavonoids, saponins, tannins, terpenoids, proteins and anthraquinone. *Syzygium samarangense* produces diverse metabolites, including flavonoids, phenolic compounds, resorcinol derivatives, acylphloroglucinols, tannins, terpenoids, sterols, and other metabolite groups. The present result of repellent activity showed that *Annona muricata*. Methanol leaf extract of *Annona muricata* was found as a active grain protectants. These insecticidal effects of the botanicals and potential for their local availability make them attractive candidate in upgrading traditional post-harvest potential practices. These test plants being medicinal would yield naturally sound chemicals having no harmful effects on the non-target organisms.

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