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Studies on Antibacterial and Insecticidal Activities of *Suregada multiflora*

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Abstract: The methanol: ethyl acetate (1:9) extract of the root and leaves of *Suregada multiflora* were tested against five Gram-positive and four Gram-negative bacteria. Root extract was active against all tested microbial species and the highest activity was shown against *Escherichia coli* with a zone of inhibition 13±0.01 mm. Leaves extract showed mild activity for all the tested bacterial strains (range of inhibition zone was 5±0.10 to 6±0.13 mm) except *Bacillus cereus, B. megatherium, B. anthracis and Shigella boydii*. Significant and highest minimum inhibitory concentration (MIC) values were observed by root extract against *Escherichia coli* with the value of 0.625 mg/ml. In insecticidal study, the root extract of *Suregada multiflora* showed better activity with 100% mortality rate of *Tribolium castaneum* at a dose of 50mg/ml with 12 hours and also showed the activity in a dose dependent manner, whereas the leaves extract of *Suregada multiflora* showed 40% mortality rate of *Tribolium castaneum* at a dose of 50mg/ml with 48 hours.

Key words: *Suregada multiflora* % *Tribolium castaneum* % Antibacterial % Insecticidal activity

INTRODUCTION

The history of plants being used for medicinal purpose is probably as old as the history of mankind. Extraction and characterization of several active phytocompounds from these green factories have given birth to some high activity profile drugs. The potential natural drugs like vincristine, vinblastine and taxol can be the chief examples [1]. Simultaneous with population explosion, virulent strains of microorganisms become more common and their increased attack accounts for increased mortality [2]. Bangladesh, being a country with high density of population, infectious diseases becomes a great challenge in the health and economic sector. To prevent infectious diseases, large numbers of antibiotics, mostly synthetic drugs, of different chemical nature have been developed in the last few decades. Nowadays, a good number of antibiotics are found to be microbiology resistant due to their inappropriate and injudicious uses or self treatment practices [3]. So the developments of new antibacterial agents are necessary to combat the problem of microbial resistance and for substitution with ineffective ones. Moreover, it is presumed that the broad spectrum effectiveness of plant species may provide a suitable basis for new antimicrobial therapies [4].

Higher plants are rich source of novel natural substances that can be used to develop environmental safe methods for insect control [5]. Yang and Tang [6] reviewed the plant used for insect control and found that there is a strong connection between medicinal and pesticidal plants. Not only the world wide annual losses of food grains storage caused by insects have been estimated to be about 10% of the world's production, but losses of 25% or more may also occur in tropical countries through insect attack after harvest [7]. *Tribolium castaneum* (Herbst) is considered to be a major pest of stored grains. In Bangladesh *Tribolium castaneum* is abundantly found in stored grains of different cereals.

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Control of these insects relies heavily on the use of synthetic insecticides and fumigants, which has led to problem such as disturbances of the environment, increasing cost of application, pest resurgence, resistant to pesticides and lethal effects on non-target organism in addition to direct toxicity to users [8, 9]. So there is an immediate need to develop eco-friendly alternatives with low cost and easy to use.

Suregada multiflora (syn. *Gelonium multiflorum* A. Juss) belongs to the family Euphorbiaceae, grows in the tropical and the subtropical areas of Asia and Africa. It is locally known as false lime and used traditionally for the treatment of hepatic and gum diseases [10]. In Thai traditional medicine, the bark of this plant has been used to treat hepatitis, lymphatic disorders, skin diseases, venereal diseases, fungal infections and leprosy. Wood parts have been used for treatment of pyrexia, eczema and venereal diseases, whereas the roots have been used for treating skin infection and lymphatic disorders [11]. Previous phytochemical investigations on some species of this genus have resulted in the isolation of flavonoids [12, 13], triterpenoids [14, 15] and some diterpenoids [16-20]. The crude extract (CH_2Cl_2–MeOH, 1:1) of *S. multiflora* exhibited selective cytotoxicity against different human tumor cell lines [21]. This plant has also been reported to contain anti-human immunodeficiency virus type 1 (HIV-1) protein, GAP31 and also to exhibit the inhibitory effect on the infection and replication of herpes simplex virus (HSV) [22]. But still no scientific and methodical investigation has so far been reported in the literatures regarding its antimicrobial and insecticidal action. Therefore, as a part of our ongoing phytochemical and pharmacological investigations on local medicinal plants of Bangladesh, [23-24] the present study has been designed to examine the antimicrobial and insecticidal activity of the crude extract (Methanol (MeOH): Ethylacetate (EtOAc)-1:9) of the leaves and roots of *Suregada multiflora* against some pathogenic bacteria using disc diffusion method and adult *Tribolium castaneum* (Herbst) by surface film treatment, respectively.

MATERIALS AND METHODS

Plant Materials: Different parts (leaves and roots) of *S. multiflora* were collected from the adjoining area of Rajshahi University Campus, Bangladesh during the month of April 2009 and were identified by Taxonomist, Department of Botany at University of Rajshahi, Bangladesh, where a voucher specimen (Voucher No # 37) has been deposited for future references.

Preparation of Extracts: The roots and leaves of *S. multiflora* were dried in an oven at 37°C and then pulverized into course powder with a mechanical grinder, passing through sieve #40 and stored separately in an air tight container. The dried powdered materials (1.0 kg) each, extracted three times by sonication for 30 min with MeOH:EtOAc (1:9) mixture (1000ml) and then filtered.. The total filtrate of both was concentrated to dryness, *in vacuo* at 40°C to render the crude extracts 300 g and 240 g, respectively.

Screening of Antibacterial Activity: Test Microorganisms: Bacterial strains (Gram positive and Gram negative) were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). *Bacillus anthracis* ATCC 14321, *Bacillus cereus* ATCC 14579, *B. megaterium* ATCC 13578, *B. subtilis* ATCC 6059, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Shigella flexneri* ATCC 9221, *Shigella boydii* ATCC 9234, were used as test microorganisms. All these bacterial species are recommended by ATCC for their susceptibility assay. All the test strains are maintained and tested on Nutrient Agar medium (NA) for bacteria.

Antibacterial Assay: The crude extract of the root and leaves of *Suregada multiflora* were tested for antibacterial activity by disc diffusion method [25]. Both the crude extracts were separately dissolved in respective solvent (1 ml) to get a concentration of 200µg/disc. The test microorganisms were inoculated into respective medium by spread plate method with 24 h cultured bacteria, grown in nutrient agar medium. After solidification the filter paper disc (5 mm diameter) impregnated with sample, the standard antibiotic (Kanamycin-30µg/disc) as positive and as negative controls, a blank disc impregnated with 30µl respective solvent was used. The spatial arrangement discs were such that the discs were not closer than 15 mm to the edge of the plate to prevent overlapping the inhibition zone. The plates were then kept in a refrigerator at 4°C for about 24 hours in order to provide sufficient time to diffuse the sample and standard from the discs to surrounding agar medium. Finally, the plates were invested and kept in an incubator at 37°C for 24 hours. After incubation the antibacterial activity of the test material was determined by measuring the diameter of the zone of inhibition in terms of millimeters (mm) with a transparent scale and the experiment was carried out in triplicate. The extracts that showed prominent

antimicrobial activity were later tested to determine the Minimal Inhibitory Concentration (MIC) for each bacterial sample according to method [26].

Determination of Minimum Inhibitory Concentration (MIC): MIC values were also studied for microorganisms, which were determined as sensitive to the extract in disc diffusion assay. In order to determine the MIC values, extracts were dissolved in dimethyl sulfhoxide (DMSO), 10% (w/v), to make a concentration of 100 mg/ml. The extracts were diluted in a simple dilution manner to make concentrations in the range of 0.312, 0.625, 1.25, 2.5, 5, 10 and 20 mg/ml. 0.1 ml of the extracts were then added to each hole. The MIC was taken as the lowest concentration of extracts that caused a clear to semi clear inhibition zone around the hole. All the tests were repeated in triplicates.

Determination of Relative Percentage Inhibition: The relative percentage inhibition with respect to positive control was calculated applying the familiar formula [27]. Relative percentage inhibition of the test extract = [{100 x (a - b)}/(c - b)]. Where, a: total inhibition area of the test extract; b: total inhibition area of the solvent; c: total inhibition area of the standard drug. Total inhibition area was calculated according area = Br^2; where, r = radius of the inhibition zone.

Screening of Insecticidal Activity:

Collection of Test Insects: Insect's *Tribolium castaneum* used in the present experiment were provided from the stock cultures of the Crop Protection and Toxicology Laboratory, University of Rajshahi, Bangladesh.

Insecticidal Assay: To conduct surface film activity test 60 mm petri dishes were taken for all extracts and their replication. Each extract (50 mg) was dissolved into 1ml respective solvent. Then they were poured into the lower part of the petri dish and allowed them to dry out. Then insects were released in each of the treated petri dish. A control experiment applying only the solvent into the petri dish, was also set at the same time under the same conditions [28]. After completing all the arrangements, treated petri dishes were placed in a secured place at room temperature. The whole experiment was observed from time to time and mortality was observed first after 30 minutes from the start and then after 48 hrs of exposure and the data was recorded. A simple microscope was used to check each and every beetle by tracing natural movement of its organs. In some cases hot needle was taken closer to the bodies (without movement) to confirm death. Attention was also paid to recover the insects if occurred. The mortality records of the *Tribolium castaneum* adults were corrected by the Abbott's formula [29].

$$P_r = (P_0 – P_c \setminus 100 - P_c) \times 100$$

Where,

P_r = Corrected mortality%; P_o = Observed mortality%; P_c = Control mortality%, sometimes called natural mortality%

Statistical Analysis: All assays were performed in triplicates under strict aseptic conditions to ensure consistency of all findings. Data of all experiments were statistically analyzed and expressed as the mean±standard deviation of three replicate experiments.

RESULT AND DISCUSSION

Table 1 shows the antibacterial activity (zone of inhibitions) of the roots and leaves of *S. multiflora*. Root extract at a dose of 200 µg/disc showed prominent activity against all the tested bacteria with the zone of inhibition range 9±0.05 to 13±0.01 mm. The highest zone of inhibition was found against *Escherichia coli* (13±0.01 mm), followed by *Bacillus subtilis* and *Shigella boydii* (zone of inhibition 12±0.05 and 12±0.07 mm, respectively) whereas the lowest activity was shown against *Pseudomonas aeruginosa* (9±0.05 mm). On the other hand, leaves extract was active against *Staphylococcus aureus, Bacillus subtilis, Shigella flexneri, Pseudomonas aeruginosa* and *Escherichia coli*, while *Bacillus cereus, Bacillus megatherium, Bacillus anthracis and Shigella boydii* showed resistance to leaves extracts.

Since root extract showed the significant activity in all the tested bacterial strains, minimum inhibitory concentration (MIC) values were applied in only root extract against susceptible bacteria (Table 1). All the tested extracts showed significant variations in MIC values, which depend upon the test bacteria. *Escherichia coli* (the most sensitive bacteria) showed the MIC value of 0.625 mg/ml.

Root extract of *S. multiflora* showed the maximum relative percentage inhibition against *E. coli* (41.23%) followed by *Shigella boydii* (34.45%) and *Bacillus subtilis* (32.12%) at the dose of 200 µg/ml. However, the relative percentage inhibition ranges was 11.21% - 14.53%, at the dose of 200 µg/ml, for *S. multiflora* leaves extract, at the same time, no relative percentage inhibition was found against *Bacillus cereus, Bacillus megatherium, Bacillus anthracis* and *Shigella boydii*.

Table 1: *In vitro* antimicrobial activity of *Suregada multiflora* root and leaves extracts

	Diameter of zone of inhibition (mm)		
Bacterial strain	[b]Std. (30µg/disc)	[a]Root extract (200µg/disc)	[a]Leaves extract (200µg/disc)
Gram positive			
Staphylococcus aureus	27±0.12	11±0.13 (2.5)	5±0.17
Bacillus cereus	24±0.10	10±0.22 (5)	NA
B.megatherium	22±0.02	11±0.02 (5)	NA
B. subtilis	25±0.22	12±0.05 (1.25)	5±0.10
B. anthracis	23±0.02	10±0.01 (10)	NA
Gram negative			
Pseudomonas aeruginosa	25±0.04	9±0.05 (20)	6±0.13
Shigella flexneri	22±0.13	11±0.12 (5)	5±0.12
Shi. boydii	24±0.17	12±0.07 (1.25)	NA
Escherichia coli	26±0.10	13±0.01 (0.625)	6±0.11

[a]Values of the observed diameter inhibition zone (mm) excluding cap diameter. Incubation conditions for bacteria – 24 hours at 37°C. Assay was performed in triplicate and results are the mean of three values±Standard Deviation. [b] Reference standard; Kanamycin. NA- Zone of inhibition < 5 mm is considered as no activity. Parenthesis indicates the MIC value (mg/ml).

Antimicrobial activities of tannins [30, 31], flavonoids [32, 33], saponins [34, 35], terpenoids [36], alkaloids [37, 38] have been documented. Previous phytochemical investigations on some species of this genus have revealed the presence of flavonoids [12, 13], triterpenoids [14, 15] and some diterpenoids [16-20]. So, the antimicrobial activity showed by extracts of *S.multiflora* (root and leaves) may be due to presence of such type of phytoconstituents.

For insecticidal activity, only root extract have shown 100% mortality rate of *Tribolium castaneum* at a dose of 50mg/ml in 12 hours. On the other hand leaves extract have shown 40% mortality rate of *Tribolium castaneum* at the dose of 50mg/ml in 48 hours. Since root extract have strong insecticidal activity, dose dependent activity was done, where 5 graded doses (viz,. 40, 30, 20, 10 and 5µg/ml) were used and the percentage of mortality were 100, 93.33, 86.66, 53.33 and 33.33% at the dose of 40, 30, 20, 10 and 5mg/ml, respectively (Fig. 1).

Table 2: Relative percentage inhibition of root and leaves extract of *S. multiflora* on various test bacterial strains

	Relative percentage inhibition (%)	
Fraction		
Gram Positive	Root extract	Leaves extract
Staphylococcus aureus	28.43	11.23
Bacillus cereus	21.56	NA
B. megatherium	27.91	NA
B.subtilis	32.12	11.68
B. anthracis	22.19	NA
Gram Negative		
Pseudomonas aeruginosa	16.45	11.95
Shigella flexneri	27.78	11.21
Shi. boydii	34.45	NA
Escherichia coli	41.23	14.53

Some *Aristolochia* species were tested for their insecticidal activity. For example, acetone and ethanol extracts of the tubercula and several compounds isolated from *Aristolochia pubescens* L. are potential botanical insecticidal agent for the control of *Anticarsia gemmatalis* L. larvae. They inhibit larval growth and induce malformed adults [39]. *Aristolochia* genus is a rich source of aristolic acid which is unique to this genus, also terpenoids [40]. On the other hand, the carbohydrates, saponins, phytosterol, phenol, flavonoids and tannins are having mosquito larvicidal activity [41]. Probable existence of flavonoids, terpenoids as well as other secondary metabolites of plant may explain the toxic effects on the studied insects.

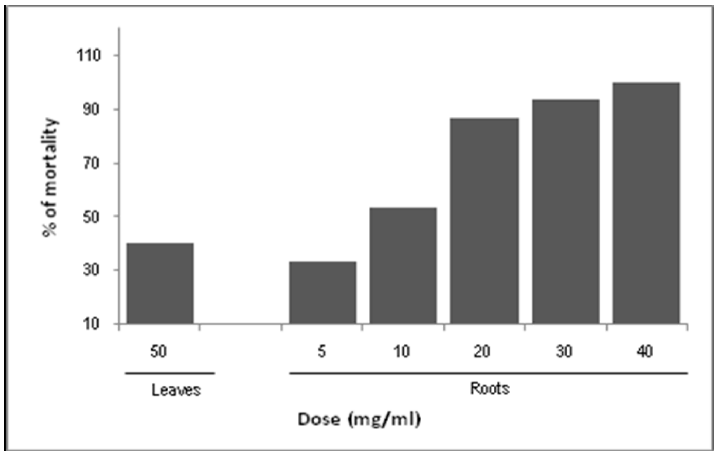

Fig. 1: Insecticidal effects of roots and leaves extracts of *S. multiflora* on *Tribolium castaneum*

In conclusion, the results of the present study are in agreement with those obtained by Khanna and Kannabiran [41] and indicated that the root extract of *S. multiflora* exhibited more insecticidal and antimicrobial properties than the leaves extract. These results not clarity which chemical compounds are responsible for the aforementioned activities. Now, the extended investigate the isolation and structure determination of the lead compound liable for aforementioned activity from this plant and work is going on in this respect in our laboratory.

REFERENCES

1. Huie, C.W., 2002. A review of modern sample preparation techniques for the extraction and analysis of medicinal plants. Anal. Bioanal. Chem., 373: 23-30.
2. Manisha, V., S. Neha and S. Satish, 2009. Antimicrobial activity of stem bark extracts of *Nyctanthes arbortristis* Linn. (Oleaceae). Int. J. Pharmacog. Phytochem. Res., 1(1): 12-14.
3. Alanis, A.J., 2005. Resistance to Antibiotics: Are We in the Post-Antibiotics Era? Arch. Med. Res., 36: 697-705.
4. Kaushik, P., 2003. Haridra (Turmeric): antibacterial potentials. Chowkhamba Sanskrit Series office, Varanasi, pp: 16.
5. Arnason, J.T., B.J.R. Philogene and P. Morand, 1989. Insecticides of plants origin. American Chemical Society Symposium Series Vol. 387. Washington, pp: 142.
6. Yang, R.Z. and C.S. Tangs, 1998. Plants used for pest control in China: a literature review. Econ. Bot., 42: 376-406.
7. Howe, R.W., 1965. Losses caused by insects and mites in stored foods and foodstuffs. Nutr. Abstr. Rev., 35: 285-302.
8. Jembere, B., D. Obeng-Ofori, A. Hassanali and G.N.N. Nyamasyo, 1995. Products derived from the leaves of *Ocimum kilimand scharicum* (labiatae) as post-harvest grain protestants against the infestation of three major stored product insect pests. Bull. Entomol. Res., 85: 361-367.
9. Okonkwo, E.U. and W.I. Okoye, 1996. The efficacy of four seed powders and the essential oils as protectants of cowpea and maije grain against infestation by *Collosobruchus maculatus* (Fabricius) Colcoptera: Bruchidae) and *Sitophilus zeamais* (Mots chulsky) (Coleoptera: Curculionitae) in Nigeria. Intl. J. Pest Management, 42: 143-146.
10. Rastogi, P. and B.N. Mehrotra, 1999. Compendium of Indian Medicinal Plants, Drug research perspective, CDRI Lucknow and NISCOM, New Delhi, 2: 1-859.
11. Wutthithamavet, W., 1997. Thai Traditional Medicine, revised edition. Odean Store Press, Bangkok, Thailand, pp: 155.
12. Parween, N. and N.U. Khan, 1987. Luteolin 7, 4'-dimethyl ether 3'-glucoside from *Gelonium multiflorum*. Phytochem., 26: 2130-1.
13. Das, B. and A.K. Chakravarty, 1993. Three flavone glycosidases from *Gelonium multiflorum*. Phytochem., 33: 493-6.
14. Sengupta, P. and H.N. Khastigir, 1963. Terpenoids and related compounds-III: bauerenol and multiflorenol from *Gelonium multiflorum* A. Juss. The structure multiflorenol. Tetrahedron, 19: 123-32.
15. Venkatesan, M., M.B. Viswanathan, N. Ramesh and P. Lakshmanaperumalsamy, 2005. Antibacterial potential from Indian *Suregada angustifolia*. J. Ethnopharmacol., 99: 349-52.
16. Talapara, B., G. Das, A.K. Das, K. Biswas and S.K. Talapatra, 1998. Sterostructures and conformations of four deterpene lactones from *Gelonium multiflorum*. Phytochem., 49: 1353-9.
17. Jahan, I.A., N. Nahar, M.M. Zaman, F. Shaheen, Z. Parween, A.U. Rahman and M.I. Choudhary, 2002. Novel diterpene lactones from *Suregada multiflora*. J. Nat. Prod., 65: 932-4.
18. Jahan, I.A., N. Nahar, M.M. Zaman, F. Shaheen, Z. Parween, A.U. Rahman and M.I. Choudhary, 2002. Six new diterpenoids from *Suregada multiflora*. J. Nat. Prod., 67: 1789-95.
19. Choudhary, M.I., H.Y. Gondal, A. Abbaskhan, I.A. Jahan, M. Parvez and N. Nahar, 2004. Revisiting diterpene lactones of *Suregada multiflora*. Tetrahedron, 60: 7933-41.
20. Cheenpracha, S., O. Yodsaoue, C. Karalai, C. Ponglimanont, S. Subhadhirasakul and S. Tewtrakul, 2006. Potential anti-allergic ent-kaurene diterpenes from the bark of *Suregada multiflora*. Phytochem., 67: 2630-4.
21. Lee, C.L., F.R. Chang, P.W. Hsieh, M.Y. Chiang, C.C. Wu and Z.Y. Huang, 2008. Cytotoxic ent-abietane diterpenes from *Gelonium aequoreum*. Phytochem., 69: 276-87.
22. Bourinbaiar, A.S. and S.L. Huang, 1996. The activity of plant-derived antiretroviral proteins MAP30 and GAP31 against herpes simplex virus infection *in vitro*. Biochem. Biophys. Res. Commun., 219: 923-929.

23. Rahman, M.H., M.B. Alam, N.S. Chowdhury, M.K. Jha, M. Hasan, M.M. Khan, M.S. Rahman and M.E. Haque, 2011. Antioxidant, analgesic and toxic potentiality of *Stephania japonica* (Thunb.) Miers. Leaf. Int. J. Pharmacol., 7(2): 257-262.
24. Alam, M.B., M.S. Hossain and M.E. Haque, 2010. Evaluation of antimicrobial and toxicity of different fractions of *Dillenia indica* linn. bark extract. J. Global Pharma Tech., 2(11): 37-42.
25. Olurinola, P.F., 1996. A laboratory manual of pharmaceutical microbiology. National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria, pp: 69.
26. Singh, M., R. Govindarajan, V. Nath, A.K.S. Rawat and S. Mehrotra, 2007. Antimicrobial, wound healing and antioxidant activity of *Plagiochasma appendiculatum* Lehm, et Lind. J. Ethnopharmacol., 107: 67-72.
27. Ajay, K.K., R.M.K. Lokanatha and K.B. Umesha, 2002. Evaluation of antibacterial activity of 3, 5-dicyano-4,6-diaryl-4-ethoxycarbonyl-piperid-2-ones. J. Pharm. Biomed. Ana., 27: 837-840.
28. Bousquet, Y., 1990. Beetles associated with stored products in Canada. Canadian Government Publishing Centre, Ottawa, pp: 189-192.
29. Abott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
30. Doss, A., H.M. Mubarack and R. Dhanabalam, 2009. Pharmacological importance of *Solanum trilobatum*. Ind. J. Sci. Tech., 2(2): 41-43.
31. Ho, K.Y., C.C Tasi, J.S. Huang, C.P. Chen, T.C. Lin and C.C. Lin, 2001. Antimicrobial activity of tannin components from *Vaccinium vitis- idaea* L. J. Pharm. Pharmacol., 53(2): 187-191.
32. Mandalari, G., R.N. Bennett, G. Bisignano, D. Trombetta, A. Saija, C.B. Faulds, M.J. Gasson and A. Narbad, 2007. Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a by product of the essential oil industry. J. App. Micro., 103(6): 2056-2064.
33. Pepeljnjak, S., Z. Kalodera and M. Zovko, 2005. Antimicrobial activity of flavonoids from *Pelergonium radula* (Cav.) L' Herit. Acta Pharma., 55: 431-435.
34. Avato, P., R. Bucci, A. Tava, C. Vitali, A. rosato, Z. Bially and M. Jurzysta, 2006. Antimicrobial activity of saponins from *Medicago sp*.: structure activity relationship. Phytother. Res., 20(6): 454-457.
35. Baharaminejad, S., R.E. Asenstorfer, I.T. Riley and C.J. Schultz, 2007. Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots of Oats (*Avena sativa* L.). J. Phytopathol., 156(1): 1-7.
36. Fnato gawa, K., S. Hayashi, H. Shimomura, T. Yoshida, T. Hatano, H. Ito and Y. Hirari, 2004. Antimicrobial activity of hydrolysable tannins derived from medicinal plants against *Helicobacterp ylori*. Microbiol. Immunol., 48(4): 251-261.
37. Navarro, V. and G. Delgado, 1999. Two antimicrobial alkaloids from *Bocconia arborea*. J. Ethnopharmacol., 66(2): 223-6.
38. Faizi, S. and R.A.T. Ahmed, 2003. New antimicrobial alkaloids from the roots of *Polyalthia longifolia* var. *pendula*. Planta Med., 69(4): 350-355.
39. Nascimento, I.R., A.T. Murata, S.A. Bortoli and L.M. Lopes, 2004. Insecticidal activity of chemical constituents from *Aristolochia pubescens* against *Anticarsia gemmatalis* larvae. Pest Manag. Sci., 60: 413-6.
40. Wu, T.S., A.G. Damu, C.R. Su and P.C. Kuo, 2004. Terpenoids of *Aristolochia* and their biological activities. Nat. Prod. Rep., 21: 594-624.
41. Khanna, V.G. and K. Kannabiran, 2007. Larvicidal effect of *Hemidesmus indicus, Gymnema Sylvestre* and *Ecliptaprostrata* against *Culex qinquifaciatus* mosquito larvae. Afr. J. Biotech., 6(3): 307-311.