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Preliminary Analysis of Insecticidal and Antimicrobial Potential of *Andrographis paniculata*

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Abstracts: Bioactive products of plant origin have been in practice to treat various diseases and food protection. The insecticidal and antimicrobial effects of leaf extracts of *Andrographis paniculata* (Family-*Acanthaceae*), prepared by the hot-wet extraction method using five solvents, were studied on the stored-grain insect, *Tribolium castaneum* (Herbst) and four aquatic pathogens. Statistical comparative analyses of the mortality rate of insects were evaluated concerning increasing concentration as well as for the study of antibacterial activity. The *iso*-propanolic extract showed a maximum lethal effect (57 %) on the insect as compared to other solvent extracts. The LD_{50} value of propanolic extract after 10 days of treatment was 63.27 mg/g. On the other hand, the aqueous (82 %) and *iso*-propanolic extracts exhibited a significant antibiosis effect. The gram-negative pathogens *A. hydrophila* and *V. parahaemolyticus* showed selective susceptibility towards different solvent extracts compared to the high susceptibility effect of gram-negative *P. fluorescens* and gram-positive *Streptococcus sp.* The present findings support the potential of *A. paniculata* as a natural alternative and suggest the preferred choice of solvents for the extraction of specific phytoactive compounds with insecticidal and antibiotic potential.

Key words: Phytochemicals, insecticides, *T. castaneum,* antibiosis, aquatic pathogens, *A. hydrophila, P. fluorescens*.

Introduction

The quadrupling of the global population has automatically increased the demand for food which is expected to increase between 59 % - 98 % by 2050 [1]. Similarly, the escalation in consumption of cereals and wheat is estimated to rise at 1.4 % per year and thereby creating a deficit of 265 million tons in developing countries. In addition to this, enhancing productivity is challenged by climate change, rapid urbanization, and food loss, especially during the post-harvest period. Various estimates suggest that pests/ insects are primarily responsible for about 20 % grain damage during storage. Especially in developing countries near the tropics, this can reach up to 50 % during high humidity and warmer seasons [2]. The red flour beetle, *Tribolium castaneum*, is one of the major insects of the destruction of wheat grains during storage. On the other hand, the aquaculture products, consumed in a substantial quantity, need proper management for sustained food productivity which is challenged hindered by the unexpected deaths of fishes by pathogenic bacteria. In general, these challenges are mitigated by the application of synthetic insecticides and/ or disinfectants and antibiotics. However, synthetic

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pesticides or aquatic disinfectants not only have harmful effects on humans or non-target species but also induce pesticide resistance in beetles [3] and antibiotic resistance in aquatic pathogens [4], respectively. Therefore, there is a quest for developing eco-friendly alternatives against insects or aquatic pathogens.

Herbal extracts are now gaining popularity and studies have been pursued to characterize their bioactive compounds with a scope of application in agriculture and pharmaceutics. The native herbaceous plant *Andrographis paniculata* (Burm.f.) of family *Acanthaceae*, commonly known as "King of Bitters", have been established as a natural medicine for the treatments of various human ailments [5]. Moreover, bioactive studies of this plant extracts on diamondback moth, *Plutella xylostella* [6] have indicated the possession of insect-specific active principles. Similarly, the antibiotic roles of *A. paniculata* plant extract on human pathogens [7,8] have become the basis to study the effects on aquatic pathogens. The present study was undertaken to evaluate the mortality and deterrence potential of different solvent extracts of the leaves of *A. paniculata* against the insect *T. castaneum* and four aquaculture pathogens.

Materials and methods

Extraction and estimation of plant extracts

Preparation of plant samples and soxhlet extraction

The plant, *Andrographis paniculata,* samples were collected locally and flowering specimens were identified at Regional Plant Resource Center (RPRC), Bhubaneswar, India. The shade dried and ground (20 g) leaf samples of *A. paniculata* were subjected soxhlet extraction by five solvents with their increasing polarities such as petroleum ether (PE)(1), ethyl acetate (EA)(2), acetone (Act)(3), propanol-2 (2-Pro)(4) and distilled water (Aq)(5). The powdered samples were placed in a pre-washed cellulose thimble and extracted with 250 ml of the chosen solvent for 6-7 h having a cycle duration of 7-10 min/cycle. Subsequently, these extracts were concentrated in a rotary evaporator and used in subsequent experimental analysis.

Preliminary qualitative and quantitative phytochemicals analysis

The extracts obtained were dissolved in the corresponding pure solvent to prepare a stock solution of 10 % (w/v). The obtained stock solution was qualitatively assayed for various phytochemical constituents [9] such as (a) alkaloids, (b) anthraquinones, (c) flavonoids, (d) glycosides, (e) phenols, (f) reducing sugars, (g) saponins, (h) tannins, (i) terpenoids.

Estimation of total phenolic content

Total phenol content was determined by Folin-Ciocalteu reagent method [10]. Each crude sample (1 ml) was treated with 10 % Folin-Ciocalteu reagent and 7.5 % Na2CO3 solution followed by dark incubation of 2 hrs and measured at 760 nm. Total phenolic content (TPC) was represented as gallic acid equivalents (GAE).

Estimation of total flavonoids

The diluted plant extract solutions were processed with an equal volume of 5 % sodium nitrite and 10 % aluminum chloride with incubation of 5 min after each solution addition. Then it was followed by the addition of 2 ml of 4 % sodium hydroxide and dilution with distilled water to 5 ml and dark incubation of 15 mins at room temperature [11]. The absorbance was measured at 510 nm and estimated from the quercetin standard curve.

Estimation of total reducing sugar content

Di-nitrosalicylic acid (DNS) reagent, prepared with 2M sodium hydroxide and potassium sodium tartrate was employed to determine the total reducing sugars content. The absorbance of the colored solutions, developed after hot water bath incubation for 10 mins and dilution, was measured at 540 nm and represented as glucose equivalents.

Bioassay studies

Insect culture and rearing

The rearing of the stored-grain pest, *Tribolium castaneum* (Herbst) (*Tenebrionidae*) was performed according to suggestions of Zettler [12] and Talukder & Howse [13] with some minor modifications. In-house homogenous insect population was

achieved by repeated sieving and rearing of eggs in a fresh medium of flour and baker's yeast extract (10:1) at 28±2oC; 70±5 % RH with 12 hrs of alternating light and dark periods [14]. Insect specimens were confirmed by the entomology department, OUAT, Bhubaneswar, India. To avoid behavioral bias before experimentation, 15 days old adult *T. castaneum* were starved overnight in empty jars.

Insecticidal effects of extracts on insect

The insecticidal activities of five solvent extracts were determined by mixing solvent dissolved extracts with a sterile diet with mass concentration (w/w) of 2.5 %, 5 %, and 10 %. The treated and control (solvent only) feeds were dried for 24 hrs at room temperature (30°C). Then sets of ten pre-conditioned (starved) adults of *T. castaneum* were introduced into the respective jars, covered with the muslin cloth. A different batch of treatments was observed after 10 days of exposure to evaluate the percentage of mortality for different dosages.

Contact toxicity of extracts on insect

The residual film bioassay technique was used to study the contact toxicity of extracts [15]. Different dosages of extracts were applied on the grains and air-dried which was then placed at the bottom of a petri-dish. The Petri-dishes were sealed with parafilm and the mortality effect on insects was recorded after a regular interval.

Antibacterial bioassays of plants extracts

The four aquaculture pathogens *viz.*, *Aeromonas hydrophila, Pseudomonas fluorescens, Vibrio parahaemolyticus,* and *Streptococcus* sp. were collected from Fish Health Management Division, Central Institute of Freshwater Aquaculture, Bhubaneswar and were cultured for the antimicrobial assays. Antibacterial action of the *A. paniculata* leaf extracts was explored by disc diffusion assay [16]. Dried and sterilized filter paper discs (4 mm diameter) soaked with *A. paniculata* solvent extracts of 60 µg/disc were placed on the nutrient agar medium which was already spread with the test pathogen and incubated at 37°C for 18-20 hours. The antimicrobial potency of the extracts was represented as a zone of inhibition in millimeters. Simultaneously each plate was also tested with negative control and positive control containing only dissolving solvent and tetracycline (20 µg/disc) respectively. The antibacterial activity was evaluated by calculating the percentage of relative inhibition zone diameter (% RIZD) as follows:

$$\% \text{ RIZD} = (\text{IZD sample} - \text{IZD negative control} / \text{IZD antibiotic standard}) \times 100$$

Where IZD is the diameter of the inhibition zone (mm) and were expressed as mean ± SD.

Statistical analyses

The susceptibility tests and percentage mortality were statistically analyzed by univariate ANOVA and regression analysis at the significance level of p=0.05 and confidence interval of (CI) 95 %, using IBM-SPSS Software. The lethal doses (LD_{50}) of each extract fractions i.e to kill 50 % of adults were calculated by Probit analysis. The Inhibition Zone Diameter data for all concentrations were subjected to one-way analysis of variance at $P < 0.05$.

Results and discussion

Preliminary biochemical evaluation

Naturally occurring molecules in plants have a different effect on their biotic and abiotic niche. The plant parts of *A. paniculata*, especially leaves have been traditionally practiced as a folklore medicine in Asia [5]. Due to their differential solubility property, metabolites or bioactive compounds in plant parts were extracted in solvents of increasing polarity. The soxhlet extract of aqueous fraction showed a maximum mass percentage weight (16.75 %) compared to other non-polar solvents.

Conventional phytochemical analysis indicated (Table 1) the presence of alkaloids, flavonoids, glycosides, saponins, and reducing sugar in all solvent extracts of leaf samples. The anthraquinone and tannin component was observed to be absent or very negligible presence. Quantitative investigations exhibited that aqueous fraction possessed a higher amount of flavonoids (19.91±0.08 mg), phenolics (91.39 ± 5.15 mg)

Table 1. Phytochemical estimations of soxhlet extracts of *A. paniculata*

Phytoconstituents	Test name	APL-1	APL-2	APL-3	APL-4	APL-5
Alkaloids	Wagner's test	(+)	(++)	(++)	(+)	(++)
Anthraquinone	Borntrager's test	(-)	(-)	(-)	(-)	(-)
Flavonoids	Alkaline reagent	(+)	(+)	(+)	(+)	(+)
Glycosides	Killer kalani test	(+)	(+)	(+)	(++)	(++)
Phenols	Lead acetate	(-)	(+)	(-)	(+)	(++)
Reducing sugars	Fehling test	(++)	(++)	(++)	(++)	(++)
Saponins	Frothing test	(+)	(++)	(+)	(++)	(+)
Tannins	$FeCl_3$ test	(-)	(-)	(-)	(-)	(-)
Terpenoids	Salkowski test	(-)	(+)	(-)	(-)	(+)

[Five solvent fractions were named as APL-1 for Petroleum ether (PE), APL-2 for Ethyl acetate (EA), APL-3 for Acetone (Act), APL-4 for propanol-2 (2-Pro) and APL-5 for aqueous (Aq)]

compared to other solvent extracts. The extracts of acetone, ethyl acetate, and propanol-2 were estimated to have a comparatively similar quantity for TPC (43.9 - 47.7 mg/g extract) and flavonoids (13.09 - 14.2 mg/g extract). However, the quantity of reducing sugar compounds in ethyl acetate (155.5 mg/g) is comparatively more than other solvent extracts even from the aqueous extract (122.5 mg/g).

Lethal effects on the insect *T. castaneum*

All the five solvents extracts of *A. paniculata* leaves exhibited possession of moderate to a high amount of insect-specific toxic compounds (Fig. 1). After 10 days of exposure, about 50 % of lethality was observed in iso-propanolic (56.6 ±3.7 %) and aqueous (43.4 ±5.7 %) extracts at 10 % (w/w) concentration. Even at 50 mg/ml concentration of iso-propanolic extract has significant effect (50 ±5.7 %). However, minimal or no activity was observed in ethyl acetate fraction (13.3 ± 3.7 % at higher concentration) and moderate activity by the acetone and petroleum ether extracts. Although, a significant amount of phytochemicals were observed in non-polar solvents, especially in ethyl acetate the bioassay results indicated efficient extraction of insect-specific toxic compounds in isopropanol and aqueous solvents. However, earlier studies have reported that ethyl acetate extracts of *Sphaeranthus indicus* and *Prosopis juliflora* and hexane extract of *Artemisia vulgaris* have significant insecticidal activity against *T. castaneum* [17]. The potential compounds present in the plant may leach out according to the polarity of the solvent used.

The present results showed that mortality (LD_{50}) of *T. castaneum* has a direct correlation with an increase in dosage treatment. The possible reason is that feeding of the more botanical particle along with flour particle increased with concentration increase. From the probit regression analysis, the LD_{50} values range from 63.27 mg/g (isopropanol) to 303.87 mg/g (ethyl acetate) for 10 days of the treatment period. The comparable activity was also observed in an aqueous extract with LD50 142.86 mg/g. Plant essential oils of *A. indica* (neem) are effective in controlling *T. Castaneum* [18] and *T. confusum* with LC50 value ranging from 7.39 to 19.24 mg/L [19]. A similar mortality effect against *T. castaneum* was observed in the extracts of *Zingiber officinale* rhizomes and *Allium sativum* [17,20]. Moderate to the high susceptibility of insects towards the extracts of *A. paniculata* affirms as a natural alternative to synthetic insecticidal compounds.

Contact toxicity effect on *T. castaneum*

The results demonstrated that extracts did not show potent contact toxicity against the stored grain insect. Non-lethal activity within 24 hrs in any extracts suggests negligible efficacy as a contact poison. This can be attributed that the bioactive molecules affect the insect by ingestion molecules. The probable mechanism may be due

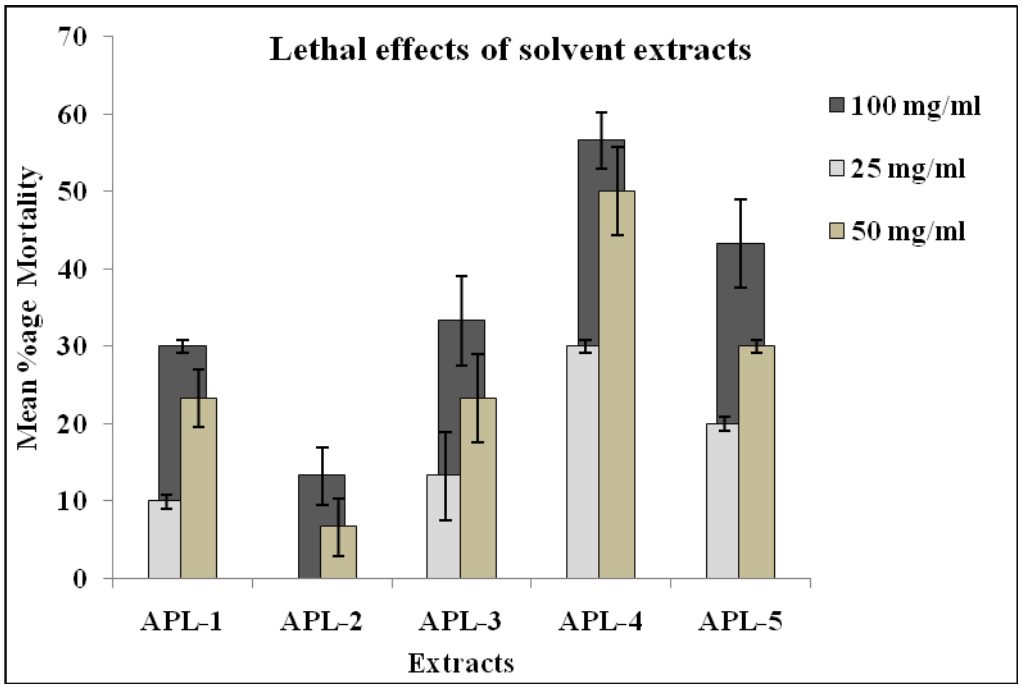

Fig. 1. Comparative representation of averaged mortality percentage of each extracts with respect to concentrations

to impairment of the digestion system of insects, such as nicotine of *Nicotiana tabacum* which acts as strong contact-stomach poison [21]. Due to the bio-degradability of the products, botanicals are considered as an alternative in managing stored grain pests. Our study supports the screening of insecticidal compounds from plants, as plants are a huge reservoir of diverse bioactive compounds.

Antimicrobial activity

The *A. paniculata* plant extracts, mostly aerial parts, and leaves have antibacterial, antifungal, antiviral, choleretic, and hypoglycaemic potential [22]. The susceptibility of selected pathogenic (aquatic) bacteria were examined against different solvent extracts of leaves (Table 2) by disc diffusion assay in agar plates. The acetone and iso-propanolic extracts showed activity against all the four microbes while the petroleum ether, ethyl acetate, and aqueous extracts didn't show activity against gram-negative *A. hydrophila* at a concentration of 60 µg/disc. The comparatively similar activity of all the extracts concerning standard antibiotic was observed against gram-negative bacteria *P. fluorescens* and gram-positive bacteria *Streptococcus sp.* while maximum activity observed in the aqueous fraction. Moreover, the pathogens *A. hydrophila* and *V. parahaemolyticus* showed maximum susceptibility towards iso-propanolic and petroleum ether extracts, respectively.

Although, sizes of a clear zone in *V. parahaemolyticus* was bigger (petroleum ether) than other test microbes, the maximum %RIZD (Relative Inhibition Zone Diameter) of *V. parahaemolyticus* was 62.83±0.47 (PE extract) compared to *P. fluorescens* (80.91±1.29), *Streptococcus sp.* (82.50±1.61) for aqueous extracts and *A. hydrophila* (71.68±1.43) for 2-propanol extract. The *P. fluorescens* and *Streptococcus sp.* showed susceptibility towards all the solvent extracts of leaves of *A. paniculata* (%RIZD 66.82±4.5 to 82.50± 1.61) while significantly specific activity was observed for *A. hydrophila* (2-propanol) and *V. parahaemolyticus* (petroleum ether).

The antibacterial activity of the plant extracts is due to the antagonistic role of different secondary metabolites such as tannin, saponin, and glycosides, which occurred as variable quantity [23,24,25]. For phytochemical analysis, the significant activity of aqueous and iso-propanolic leaf extracts of *A. paniculata* can be attributed to the

Table 2. Comparative antimicrobial effect of solvent extracts of leaves of *A. paniculata*

Microorganisms	Zone of inhibition of solvent extracts in mm ± SD						
	PE	EA	Act	2-Prop	Aqu	*Tet*$^{+}$	(-) con
Aeromonas hydrophila	0.0±0.0	0.0±0.0	6.0±0.001	8.5±0.7	0.0±0.0	12.0±1.41	0
Pseudomonas fluorescens	8.5±0.70	8.0±0.001	8.5±0.7	7.0±0.001	8.5±0.7	10.5±0.7	0
Streptococcus sp.	7.5±0.7	8.5±0.70	8.0±0.001	7.5±0.7	9.0±0.001	11.0±1.41	0
Vibrio parahaemolyticus	11.0±1.41	8.0±0.001	8.5±0.7	9.5±2.12	8.0±1.41	17.5±2.1	0

[Extract conc @ 60 µg/disc, *Tet*$^{+}$- Positive Control (Tetracycline),
(-) con- Negative Control (Solvent only)]

presence of alkaloids, phenols, saponin, and terpenoids which are known as potential antimicrobial agents even if the tannin quantity is negligible (Table 1). Although, previous studies have reported that gram-positive bacteria are more susceptible to plant extracts compared to gram-negative bacteria. Our studies are partially under the above fact, as only the gram-negative *A. hydrophila* has susceptibility towards very specific extract while the gram-negative *P. fluore-scens* and *V. parahaemolyticus* have significant susceptibility towards all the solvent extracts, similar to susceptibility *Streptococcus sp*. In general, the plausible reason for variable resistance shown by gram-negative bacteria is due to the presence of an outer membrane and a unique periplasmic space which is absent in gram-positive bacteria [26,27.] Thus, *A. paniculata* leaf samples extracted in non-polar solvents (petroleum ether and acetone) as well as in aqueous medium possess an excellent antimicrobial property which can be investigated to develop potential alternate antibiotics.

Conclusion

The plant extracts can be used as a substitute to control disease outbreaks in aquaculture production as well as the management of agricultural storage insects. Observations of the present study evaluated that the plant *Andrographis paniculata,* especially iso-propanolic or aqueous extracts, could be developed into an alternative natural pesticide agent and disinfectant. This kind of screening and evaluation studies will enhance the plausibility of developing new bioactive molecules for sustained food production in agriculture and aquaculture.

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Reference

1. **Valin, H., Sands, R.D., Van der Mensbrugghe, D., Nelson, G. C., Ahammad, H., Blanc, E., Bodirsky, B., et al. (2014).** The future of food demand: understanding differences in global economic models. Agricultural Economics. 45(1): 51-67.
2. **Adams, C.A. (1998).** Features-Deterioration of Stored Feed Grains-Moisture, moulds and insects interact. Poultry International. 37(13): 32-37.
3. **Oppert, B., Guedes, R.N., Aikins, M.J., Perkin, L., Chen, Z., Phillips, T. W., Zhu, K.Y., et al. (2015).** Genes related to mitochondrial functions are differentially expressed in phosphine-resistant and-susceptible *Tribolium castaneum*. BMC Genomics. 16(1): 968.
4. **Romero, J., Feijoó, C.G., Navarrete, P. (2012).** Antibiotics in aquaculture-use, abuse and alternatives. Health and Environment in Aquaculture. Intech Open.

5. **Okhuarobo, A., Falodun, J.E., Erharuyi, O., Imieje, V., Falodun, A., Langer, P. (2014).** Harnessing the medicinal properties of *Andrographis paniculata* for diseases and beyond: a review of its phytochemistry and pharmacology. Asian Pacific Journal of Tropical Disease. 4(3): 213-222.
6. **Hermawan, W., Kajiyama, S., Tsukuda, R., Fujisaki, K., Kobayashi, A., Nakasuji, F. (1994).** Antifeedant and antioviposition activities of the fractions of extract from a tropical plant, *Andrographis paniculata* (Acanthaceae), against the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). Applied Entomology and Zoology. 29(4): 533-538.
7. **Thirumurugan, K. (2010).** Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. Steroids. 1: 7.
8. **Roy, S., Rao, K., Bhuvaneswari, C., Giri, A., Mangamoori, L.N. (2010).** Phytochemical analysis of *Andrographis paniculata* extract and its antimicrobial activity. World Journal of Microbiology and Biotechnology. 26(1): 85.
9. **Dey, P.M., Harborne, J.B. (1997).** Plant biochemistry. Elsevier.
10. **Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M. (1999).** Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology. 299: 152-178.
11. **Zhishen, J., Mengcheng, T., Jianming, W. (1999).** The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64(4): 555-559.
12. **Zettler, L.J. (1991).** Pesticide resistance in *Tribolium castaneum* and *T. confusum* (Coleoptera: Tenebrionidae) from flour mills in the United States. Journal of Economic Entomology. 84(3): 763-767.
13. **Talukder, F.A., Howse, P.E. (1993).** Deterrent and insecticidal effects of extracts of pithraj, *Aphanamixis polystachya* (Meliaceae), against *Tribolium castaneum* in storage. Journal of Chemical Ecology. 19(11): 2463-2471.
14. **Islam, M.S., Talukder, F.A. (2005).** Toxic and residual effects of *Azadirachta indica*, *Tagetes erecta* and *Cynodon dactylon* seed extracts and leaf powders towards *Tribolium castaneum*. Journal of Plant Diseases and Protection. 112(6): 594-601.
15. **Rajashekar, Y., Bakthavatsalam, N., Shivanandappa, T. (2012).** Botanicals as grain protectants. Psyche: A Journal of Entomology. 2012.
16. **Bauer, A.W. (1996).** Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology. 45: 149-158.
17. **Chaubey, M.K. (2012).** Responses of *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae) against essential oils and pure compounds. Herba Polonica. 58(3).
18. **Iqbal, J., Jilani, G., Aslam, M. (2015).** Growth inhibiting effects of three different plant extracts on *Tribolium castaneum* (Herbst) (Tenebrionidae: Coleoptera). Journal of Bioresource Management. 2(2): 6.
19. **Zaka, S.M., Iqbal, N., Saeed, Q., Akrem, A., Batool, M., Khan, A.A., Anwar, A., et al. (2019).** Toxic effects of some insecticides, herbicides, and plant essential oils against *Tribolium confusum* Jacquelin du val (Insecta: Coleoptera: Tenebrionidae). Saudi Journal of Biological Sciences. 26(7): 1767-1771.
20. **Epidi, T.E., Odili, E.O. (2009).** Biocidal activity of selected plant powders against *Tribolium castaneum* Herbst in stored groundnut (*Arachis hypogaea* L.). African Journal of Environmental Science and Technology. 3(1): 001-005.
21. **Rahman, M.M. (1990).** Some promising physical, botanical and chemical methods for the protection of grain legumes against bruchids in storage under Bangladesh conditions. Bruchids and Legumes: Economics, Ecology and Co-evolution. (pp. 63-73). Springer.

22. **Akbar, S. (2011).** *Andrographis paniculata*: a review of pharmacological activities and clinical effects. Alternative Medicine Review. 16(1): 66-78.
23. **Oyekunle, M.A., Aiyelaagbe, O.O., Fafunso, M.A. (2006).** Evaluation of the antimicrobial activity of saponins extract of Sorghum bicolor *L. Moench*. African journal of Biotechnology. 5(23): 2405-2407.
24. **Nwogu, L.A., Igwe, C.U., Emejulu, A.A. (2008).** Effects of *Landolphia owariensis* leaf extract on the liver function profile and haemoglobin concentration of albino rats. African Journal of Biochemistry Research. 2(12): 240-242.
25. **Panathula, C.S., Mahadev, M.D., Naidu, C.V. (2014).** Phytochemical Investigation and *in Vitro* Antimicrobial Activity of *Centella asiatica* (L.) Urban. A Potent Anti-jaundice Medicinal Plant. International Journal of Phytomedicine. 6(2): 195.
26. **Padam, B.S., Tin, H.S., Chye, F.Y., Abdullah, M.I. (2012).** Antibacterial and Antioxidative Activities of the Various Solvent Extracts of Banana (*Musa paradisiaca* cv. Mysore) Inflorescences. Journal of Biological Sciences. 12(2): 62-73.
27. **Duffy, C.F., Power, R.F. (2001).** Antioxidant and antimicrobial properties of some Chinese plant extracts. International Journal of Antimicrobial Agents. 6(17): 527-529.