Innovations

Phytochemical Profile of Piper Guineense Seeds, Rosemarinus Officinalis Leaves and Syzygium Aromaticum Buds used in the Control of Selected Pests of Stored Grains

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***Abstract** : Piper guineense, Rosemarinus officinalis and Syzygium aromaticumare botanicals with insecticidal properties which have desirable qualities for the control of Sitophilus zeamais, Callosobruchus maculatus and Tribolium castaneum pests of stored products. Analysis of phytochemical composition of seeds from P. guineense, leaves from R. officinalis and buds from S. aromaticum were carried out. The qualitative analysis of the three plants indicates the presence of saponins, steroids, coumarin, glycosides, flavonoids, triterpenes, alkaloids and terpenoids. Flavonoid has the highest quantity in seeds of P. guineense (398.26 μg quercetin equivalent/g extract). The phenolics had the highest quantity in Rosemarinus officinalis and Syzygium aromaticum respectively. It is evident that each of the phytochemicals contributed to the activity of the botanicals in the control of the storage pests of maize, cowpea and sorghum. They exhibited high antioxidant activity.*

***Keywords**: Piper guineense, Rosemarinus officinalis, Syzygium aromaticum, Phytochemical, Insecticidal, Botanical, storage pests.*

Introduction

The importance of plants to man, his animals and environment cannot be overemphasized. Man has continued to put plants into various uses either in the form of herbs, food, fabrics and for beautification.

The use of synthetic pesticides in crop protection programs around the world has resulted in disturbances of the environment, pest resurgence, pest resistance and lethal effect to non-target organism in the agro-ecosystem in addition to direct toxicity to users (Dawodu et al., 2021a; Tudi et al., 2021). Reports of the use of plants in form of powders, oils, essential oils, extracts, wholesome and as drugs to treat man's illness and to protect his food from pests has been documented (Asha et al., 2024; Kamyab et al., 2021; Bandar et al., 2022; Bailey et al., 2022). Acceptability rate of the use of plants

for the treatments of diseases in man has gained a worldwide recognition (Zheng et al., 2020; Ashraf et al., 2022; Arunsi et al., 2022).
World Health Organization reported the need for access to traditional and complementary medicine; its integration into healthcare systems worldwide and for the protection of crops in storage through the use of plant as a source (WHO, 2024). Food and Agriculture Organization of the United Nations documented various methods of boosting farmer productivity through crop protection especially with the use of plants with insecticidal properties (FAO, 2024). Similarly, most of these plants with insecticidal qualities are used in the control of some insect pests which damage our crops. Studies on the use of insecticidal plants for the control of crop pest has been reported (Babarinde et al., 2021; Dawodu et al., 2021b; Dawodu, 2022; Babarinde et al., 2022; Dawodu et al., 2024).Efficacies of powder, essential oils and mineral oils from Piper guineense, Tephrosia vogelii, Chinopodiumam brosioides, Tithonia diversifolia, Lippia javanica, Vernonia amygdalina, Ocimum gratissimum, Rosemarinus officinalis, Sygyzium aromaticum against stored product insects have been investigated and confirmed by many workers (Dawodu et al., 2024; Babarinde et al., 2022; Safar et al., 2022).
There are certain phytochemicals in plants which are harmful to pest when consumed. These active plant chemicals maybe processed by pests in different ways such that the same allemone may have different effects on different pests. According to Barros, 2010 the active ingredient in Anadenanthera macrocarpa are alkaloids which are active against Spodoptera frudipedra (armyworm) a pest that attacks maize, rice, soybean, cotton and sorghum. It acts on the reproductive system of the pest. Malva silvertris contain napthaquinones that act on the digestive system of the pest (Knnak et al., 2012). Alkaloids found in Nicotinana tabacum act on the respiratory system (Lisa, 2014) while that found in Ryania speciosa acts on the muscles (Khater, 2012). The aim of this study is to determine the antioxidant potential, class of phytochemicals present in these plants and quantify the phytochemicals.

Methodology

Collection and Preparation of Sample

Seeds of Piper guineense, leaves of Rosemarinus officinalis and buds of Syzygium aromaticumwere used for this research. Samples of these plants were collected from Forest Research Institute of Nigeria (FRIN), Idi Ishin, Jericho in Ibadan, Nigeria. The samples were authenticated at the Herbarium of the institute. Further authentication of the plant materials was carried out at the Department of Plant Science, Bamidele Olumilua University of Education, Science and Technology, Ikere- Ekiti, Ekiti State, Nigeria.
The plant materials were dried under ambient laboratory condition for two weeks until samples obtained a constant weight. Dried samples were separately ground into

powder using kitchen mill. Each powder was kept in a clean plastic container with tightly fitted lids until use.

Extraction

Extracts from powders of P. guineenses, R. officinalis and S. aromaticum were separately prepared by soaking 500g of each plant materials in 1liter of ethanol under room temperature, for 48 hours, extracts were obtained using Soxhlet extractor and subsequently concentrated by distillation and evaporated to dryness and stored in a cool dry container until use.

Phytochemical Screening

Chemical test was carried out on the plant samples using standard procedures described by Odebiyi and Sofowora 1978.

Test for Saponins

Frothing tests: 2 cm^3 of the extract in a test tube was vigorously shaken for 2 minutes. Frothing indicates the presence of saponins.

Test for Tannins

1 cm^3 of freshly prepared 10% KOH was added to 1 cm^3 of extract in a test tube. The occurrence of dirty white precipitate indicates the evidence for the presence of tannins.

Test for Phenolics

1 drops of 5% $FeCl_3$ was added to 1 cm^3 of the extract in a test tube. A greenish precipitate emerged. This indicates the positive test for the presence of phenolics.

Test for Alkaloids

1 cm^3 of 1% HCl was added to 3 cm^3 of the extract in a test tube. The mixture was heated for 20 minutes, cooled and filtered. Thereafter two drops of Wagner's reagent was added to 1 cm^3 of the filterate, a reddish brown precipitate evolved which indicated the presence of alkaloids.

Test for Steroids

Salakowsti test. 5 drops of concentrated $H_2SO_4$was added to 1 cm^3 of the extract. Red coloration indicates the presence of steroids.

Test for Tripterpenes

5 drops of acetic anhydride was added 1 cm^3 of the extract. A drop of concentrated H_2SO_4 was then added to the mixture and steamed for 1 hour. This was followed by the addition of chloroform. A bluish green color indicates the presence of triterpenes.

Test for Phlobatannins

1 cm^3 of the extract was added to 1% HCl. A red precipitate indicated the presence of Phlobatannins.

Test for Glycosides

10 cm^3 of 50% H_2SO_4 was added to 1 cm^3 of the extract, the mixture was heated in boiling water for 15 minutes. 10 cm^3 of Fehling's solution was added and the mixture boiled. A brick red precipitate evolved which indicated the presence of glycosides.

Test for Flavonoids

1 cm^3 of 10% NaOH was added to 3 cm^3 of the extract. A yellow colouration was observed which indicates the presence of flavonoids.

Test for Phytosterols

(Finar, 1986): Liebermann-Burchard test:

50 mg of the extract was dissolved in 2 ml acetic anhydride. Two drops of concentrated H_2SO_4 were added slowly along the sides of the test tube. An array of color changes was observed which shows the presence of phytosterols.

Test for Oils and Fats (Kokate, 1999).

A small quantity was processed between two filter papers, oil stain was noticed on the paper which indicates the presence of fixed oils.

Test for Terpenoids

5ml of aqueous extract of the sample was mixed with 2 ml of $CHCl_3$ in a test tube, 3 ml of concentrated H_2SO_4 was carefully added into the mixture to form a layer. A reddish-brown coloration showed the presence of terpenoids.

Test for Amino acid (Yasuma and Ichikawa, 1953).

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was added to 2 ml of aqueous filtrate. A characteristic purple color was observed which indicated the presence of amino acids.

Estimation of total phenolic content

The total phenolic content of sample was estimated according to the method of Makkar et al (2007). The aliquots of the extract was taken in test tube and made up to the volume of 1ml with distilled water. Then 0.5ml of Folin-Ciocalteau reagent (1:1 with water) and 2.5ml of Sodium carbonate solution (20%) were added sequentially to the test tube. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40 minutes and the absorbance was recorded at 725 nm against the reagent blank. Using Gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1 -10 μg/ml. using the standard curve, the total phenolic content was calculated as Gallic acid equivalent in Mg/g of extract.

Estimation of Total Flavonoid content

Total flavonoid content was measured by aluminium chloride colorimetric assay. 1 ml of extract and standard solution of Quercetin (500 μg/ml) was added together in a 50 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3ml of 10 % $AlCl_3$ was added. 6 minutes later, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml of distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 410 nm. Total flavonoid content of the flower was expressed as percentage of Quercetin equivalent per 100 g of fresh mass.

DPPH radical assay

1 ml of various concentrations of the extract in methanol was added to 4 ml of 0.1 mmol L^{-1} methanolic solution of DPPH. A blank probe was obtained by mixing 4 ml of 0.1mmol L^{-1} methanolic solution of DPPH and 200 μl of deionized water. After 30 mins of incubation in the dark at room temperature, the absorbance was read at 517nm against the prepared blank. Inhibition of free radicals by DPPH in percentage (%) was calculated using this formula:

% inhibition = 100-(ABS Sample-ABS Blank Control) X 100.

Results

The screening of phytochemicals in extracts from P. guineense R. officinalis and S. aromaticum showed the presence of Saponin, Steroids, coumarin, glycosides, Flavonoids, Terpenoids, triterpenes and Alkaloids but absence of tannins and Phenolics was recorded in P. guineense while Anthocyanin, phlobatannin and amino acids were absent in all three extracts from P.guineense, R. officinalis and S. aromaticum as presented in table 1.

Table 1: Qualitative phytochemical analysis of secondary metabolites from Piper guineense, Rosemarinus officinalis and Sygyzium aromaticum

Phytochemicals		**Plant Species**	
	P. guineense	**R. officinalis**	**S. aromaticum**
Saponins	+	+	+
Tannins			
Phenolics	+	+	+
Steroids	+	+	+
Coumarin	+	+	+
Glycosides	+	+	+
Flavonoids	+	+	+
Triterpenes	+	+	+
Anthocyanins			
Phlobatannins			
Alkaloids	+	+	+
Terpenoids	+	+	

Keys: + Present; - Absent

Table2: Quantitative phytochemical analysis of secondary metabolites from P. guineense, R. officinalis and S. aromaticum

Sample	Flavonoids µg/g	Phenolics µg/g	Glycosides µg/g	Alkaloids µg/g	Triterpenes µg/g	Tannins µg/g	Saponins µg/g	Terpenoids µg/g	Coumarins µg/g	Steroids µg/g
P.G. Seed	398.63 ±5.53	-	7.07 ±0.17	25.62 ±0.14	124.44 ±0.46	-	0.93 ±0.02	11.45 ±0.10	54.95 ±0.23	17.09 ±± 0.21
R.O. Leaf	152.82 ±0.63	566.49 ± 0.52	12.69 ±0.32	24.27 ±0.07	106.16 ±0.02	6.83 ±0.02	0.90 ±0.02	12.86 ±0.08	76.35 ±0.08	131.62 ±0.24
S.A Bud	161.20 ± 0.33	580.88 ±0.13	12.92 ±0.02	33.71 ±0.71	167.83 ±0.17	20.50 ±0.06	0.93 ±0.02	13.23 ±0.09	56.56 ±0.1	135.53 ±0.17

Key: P.G- P. guineense, R.O- Rosemarinus officinalis, S.A - Sygyzium aromaticum

Table 3: antioxidant capacity of P. guineense, R. officinalis and S. aromaticum (%)

P. guineense	**R. officinalis**	**S. aromaticum**
72.14	75.65	78.42

Discussion

Environmental pollution as a result of persistent use of synthetic insecticides in order to control pest of crops has a grave effect on man, plants and animals. This affects both target and non-target pests leading to extermination of both beneficial and non-beneficial insects and plants. A traceable number of life-threatening diseases of man associated with direct and indirect ingestion of synthetic pesticides have been reported over the years (Rani et al., 2021; Ahmad et al., 2024) In order to reduce these damages, alternatives are sort in botanicals. Evidences have shown that pesticides developed from botanicals have been effective in the reduction and control of crop pests(Ali et al., 2022; Shai et al.,2024).These biopesticides have been found to be degradable and will naturally loose potency over a period of time after use. Botanical pesticides in this category are non-toxic to man and his environment but are always lethal to target pest. This is due to the presence of some secondary metabolites and almost no evidence of pest resurgence has been recorded, therefore is considered as good alternative to synthetic insecticides.

The presence of secondary metabolites in the plant materials is considered to be responsible for the insecticidal activity for the control of storage pests. This has prompted the phytochemical analysis of P. guineese, R. officinalis and S. aromaticumin order to identify and determine the class and types of compounds present in these botanical samples. Phytochemical analysis of the extracts from P. guineense seeds, R. officinalis leaves and S. aromaticum buds as presented in Table 1 show a strong presence of Saponins, Steroids, Coumarin, glycosides, flavonoids, triterpenes, alkaloids and terpenoids while Anthocyanin, Phlobatannins and amino acids were absent in the ethanolic extracts which was corroborated by Kalinda and Rioba, 2020; Isikhuemen et al., 2020; Lone and Jain, 2022; Ohemu et al., 2024. The efficacy of the plant materials to control storage pests has been confirmed to be due to the presence of these metabolites. The combination of the activity of these metabolites has proven to be effective in the control of Callosobruchus maculatus, Sitophilus zeamais and Tribolium castaneum (Adegbola et al., 2023; Dawodu et al., 2024).The pesticidal efficacy of the plant extracts depends on the presence of the required quantity and nature of the phytochemical present in the crude extract. In the evaluation of the total phenolic and flavonoid contents, Folin-Calcelteau reagent was employed and Gallic acid was used as the standard phenolic while aluminum colorimetric assay was used and quercetin as the standard flavonoid. The spectrophotometric quantification of the phytochemicals revealed that Piper guineense seed has the highest quantity of flavonoids while saponins showed the lowest amount as presented in table 2.The phenolics are highest in the Syzygium aromaticum buds and Rosemarinus officinalis leaf. The three plant materials studied showed appreciable quantities of flavonoids and steroids. The Syzygium aromaticum buds exhibited the highest amount in phenolics, glycosides, alkaloids, triterpenes, tannins, saponins, terpenoids and steroids while Rosemarinus officinalis leaf exhibited the highest quantity in coumarins. They all showed moderate quantity of alkaloids, triterpenes and coumarinsin antioxidant activity of some secondary metabolites identified in Tapinanthus bangwensis (Atewolara-Odule et al., 2020; Bari et al., 2021).The antioxidant capacity exhibited by the plant extracts maybe due to the presence of more than one compound. The method used in this study measured the ability to scavenge free radicals. The DPPH scavenging activity as shown in table 3 could be due to the presence of flavonoids and polyphenols.

Conclusion

Piper guineense, Rosemarinus officinalis and Syzygium aromaticum popular for their spices in the Nigerian traditional settings possess insecticidal properties which are responsible for the activity exhibited in the control of storage pests of grains. These plants have high content of polyphenols and flavonoids. They also possess moderate quantity of alkaloids, triterpenes, steroids and coumarins. However, further studies are required for the identification of compounds responsible for the pesticidal activity.

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