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# BIOLOGICAL ACTIVITIES OF CRUDE SAPONINS, METHANOLIC EXTRACT AND SUB FRACTIONS OF *TEUCRIUM STOCKSIANUM* BIOSS COLLECTED FROM NORTH WEST OF PAKISTAN

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## Abstract

Agricultural plants are the major source of income of majority population of the world. A large number of the cultivated plants are used for scientific purposes. Besides many other vital applications of plants, a parallel importance is increasing towards their research potentials. Medicinal plants are good sources of therapeutic moieties, provides safe, economical and effective remedies for different pathological conditions. In this study, methanolic extract (ME), subsequent fractions and crude saponins (CS) of *Teucrium stocksianum* (TS) have been evaluated for biological activities against various insects. The crude saponins have shown significant effect against *Heterotermes indicola* (100%, day first), *Monomorium pharaonis* (100%, at 0.4 w/v), *Tribolium castaneum* (80%) and *Rhizopertha dominica* (75%). The chloroform fraction (CF), ethyl acetate fraction (EAF) and methanolic extract (ME) demonstrated outstanding effects i.e. 84, 77.32 and 57.32% respectively while *n*-hexane (HF) and aqueous fraction (AF) showed moderate effects i.e. 57 and 54.32% respectively on day first against *H. indicola*. Similarly, on day second CF, EAF, ME, HF and AF showed 100, 92, 80, 72 and 68% lethality respectively against *H. indicola.* Moreover, all the test samples demonstrated 100% lethality on the last day against the same insects. The CF and EAF showed prominent activity against *M. pharaonis* i.e. < 0.01% (w/v) $LC_{50}$. The AF, ME and HF showed 0.26, 0.36 and 0.37% $LC_{50}$ respectively. The activity against *T.castaneum* revealed 80, 70, 65, 65, 55 and 40% lethality by saponins, CF, EAF, HF, ME and AF respectively. Tremendous effect has been shown by saponins (75%) and CF (65%), good activities have been displayed by ME (50%) and HF (45%) while EAF and AF showed moderate activities i.e. 35 and 20% respectively against *R. dominica*. Based on our findings, it could be concluded that saponins and CF were more effective against all the tested insects. These effects could be attributed to the presence of phytochemicals in the fractions that might have an insecticidal potential. Furthermore the current study has exposed that both saponins and chloroform fraction of *T.stocksianum* are the most convenient targets for the isolation of natural insecticidal compounds.

**Keywords:** *H. indicola, T. castaneum, R. dominica, M. pharaonis,* insecticidal, saponins, *Teucrium stocksianum*.

## Introduction

Agriculture is one of the major sources of income for a large number of populations of the world. The crops and forests serves a multitude role in the fulfilment of basic needs of the mankind [1-2]. The prosperity of mankind depends on agricultural outcome but some factors are there which cause the decline of agricultural production. Pests have been reported one of the prominent factors which can decrease the yield of crops [3]. These insects can either damage stems of the giant trees or destroy a vast number of crops in the fields. There is also a close possibility of these insect to damage the stored bulk of grains and to spoil or contaminate the food stuffs [4-5]. Among the wood damaging insects termites are very important. On the other hand the *pharaoh ants* are responsible for multiple types of diseases and spoilage of food materials [3, 6]. A number of efficient insecticides are available but unfortunately they have low safety profile and are hazardous to human health as they are from chemical sources [7]. Insects like *R. dominica* (grain borer) and *T. castaneum* (flour beetle) are among the group of insects causing huge destruction to grains and are most often resistant to insecticides [8-9]. The insecticides obtained from plants origin are comparatively more efficient, safer and posses negligible hazardous effects [10]

Variety of plants is used in folk medicine against various insects. The saponins obtained from most of the plants having a high potential as insecticides and anthelmintics are reported [11-13].

*Teucrium* belongs to the family Lamiaceae. The members of this genus possess diversified pharmacological potentials [14-16]. The extracts of *T.royleanum* has shown antibacterial and acetylcholine inhibition activities [17]. *T. Stocksianum* have previously been reported with strong antinociceptive potential [18]. *T.stocksianum* extract contains different classes of phytochemicals including carbohydrates, proteins, amino acids, tannins, flavonoids, terpenoids and saponins [19]. In ordered to investigate research potential of this specie, our study aims to evaluate the effect of saponins, methanolic extracts and subsequent fractions of *T.stocksianum* against different insects including *Heterotermes indicola, Monomorium pharaonis, Tribolium castaneum and Rhizopertha dominica.*

## Material and Methods

### *Collection of Insects*

The termites (*H. indicola)* were collected from the timber market located near University of Malakand, Khyber Pakhtunkhwa (KPK), Pakistan and were kept in a bottle with wooden particles. The flour beetles (*T. castaneum*) were collected from the nearby flour mill while the grain borers (*R. dominica*) were collected from the bags containing wheat grains in grocery shop. Pharaoh ants were collected from the boys hostel University of Malakand, KPK, Pakistan.

### *Collection, Extraction and Fractionation*

The plant was collected from the hilly area near University of Malakand, KPK, Pakistan. Plant was identified by plant taxonomist Dr. Muhammad Nisar Department of Botany University of Malakand Chakdara Dir, KPK, Pakistan. The plant specimen was deposited with voucher number (H.UOM.BG.199c) in the herbarium of the same University. After shade drying the plant was pulverized into fine powder. The powdered plant material (2 kg) was soaked in 80% methanol. The extract was filtered and concentrated under reduced pressure using rotary evaporator at 40 °C and obtained 150 gm of the extract. The extract was suspended in 500 ml of distilled water and transferred into a separating funnel for fractionation. Fractionation was performed using successive solvent-solvent extraction method i.e. starting from low polar solvent and continue extraction to the high polar solvent. Fractionation resulted in 26 g, 38 g, 47 g and 23 g of *n*-hexane, chloroform, ethyl acetate and aqueous fraction respectively.

### *Extraction of crude saponins*

Methanolic extract (20 g) of *T.stocksianum* was taken and added into a beaker containing equal proportion of *n*-butanol and distilled water. After thorough mixing, the whole sample was transferred from the beaker into a separating funnel with vigorous shaking. The separating funnel containing mixture was fixed in a stand till the development of two distinct layers (aqueous and *n*-butanol). The *n*-butanol layer was treated with diethyl ether yielding precipitates of saponins. The saponins was collected and dried in oven, yielded 0.6 g (3%) [20].

### *Anti termite activity*

The anti-termite assay of crude methanolic extract, saponins and subsequent fractions was

performed according to the procedure reported by Salihah *et. al.*, 1993 [21]. The plant extracts were evaluated against *H. indicola*. Sterilized filter papers having size equal to the size of the petri dishes were placed in each petri dish and treated with 2mg/ml of each extract in respective solvents. It was kept overnight to evaporate the solvent and on the next day 25 adult termites were transferred into each petri dish. The numbers of killed termites were recorded after keeping the petri dishes for 24 h at room temperature. Likewise, the results of each sample were recorded on the second and third day. Tests were performed in triplicate and the mean insects killed each day were noted. The $LC_{50}$ value of each sample was calculated using graph pad prism software (version 5.0).

***Anti-Pharaoh ant activity***

The anti-Pharaoh ant activity was conducted using the direct contact toxicity method [22]. Different concentrations i.e. 0.4, 0.6 and 0.8% (w/v) of methanolic extract, subsequent fractions and saponins of *T. stocksianum* were prepared in distilled water. Each sample was transferred to a separate petri dish containing sterilized filter paper and kept overnight until complete evaporation of the solvent from it. Pharaoh ants ($n$ = 30) were transferred into each petri dish and were kept at room temperature for 24 h. The petri dish having filter paper treated with distilled water served as control. All the tested concentrations were treated in triplicate. At 24 h, the numbers of dead and alive ants were recorded and the percent mortality was calculated using statistical tools.

***Insecticidal activity against T. castaneum and R. dominica***

In this activity the crude saponins, methanolic extract and resultant fractions of *T. stocksianum* were evaluated against *T. castaneum* (flour beetle) and *R. dominica* (grain borer). The contact toxicity method was followed in this activity [22]. Two hundred milligrams of each sample was dissolved in 3 ml of volatile solvent (methanol) to make stock solution. The sterile filter paper having the same diameter as that of the petri dishes were dipped in the stock solution and transferred into the respective labelled petri dishes. The petri dishes were kept overnight for the evaporation of organic solvent. Afterwards, 20 insects were transferred into each petri dish and were kept at room temperature for 24 h. Permethrin was used as a positive control while the volatile solvent was used as negative control. After 24 h, the numbers of killed and survived insects were observed.

***Statistics***

The results obtained were presented as mean ± SEM of 20-30 insects per group. Excel sheet was used for $LC_{50}$ values determination. Data was analysed by ANOVA followed by post hoc Dunnett's test for multiple comparison. Statistically significant values were shown as $p < 0.05$.

## Results

***Anti-termite effect***

The anti-termite effect of methanolic extract, crude saponins and subsequent fractions of *T. stocksianum* showed the highest activity for crude saponins which killed almost all the termites on the first day. The next highest observed activity was shown by chloroform fraction with 100% mortality on the second day. The lowest activity was attributed to the aqueous fraction on the day first & second as shown in Figure 1. Moreover, all the tested samples have shown 100% lethality on the third day.

***Anti-Pharaoh ants effect***

In anti-pharaoh ants activity the crude saponins showed dominant activity killing almost all the ants at lowest selected concentration. The activity of chloroform and ethyl acetate fractions were comparable showing $LC_{50}$ value of 0.01 by both Table 1. The *n*-hexane fraction was the least active showing 50.00 ±5.00, 78.33 ±2.9 and 83.33 ±2.9% mortality at concentrations 0.4, 0.6 and 0.8% (w/v) respectively shown in Figure 2.

***Insecticidal effect against T. castaneum and R. dominica***

The crude saponins was dominant among all the test samples as insecticidal against *T. castaneum* (Table 2) and *R. dominica* (Table 3) showing 80% lethality for former and 75% for the later one. Comparing the activities of all the test samples against both the insects it is clear that samples of *T. stocksianum* showed overwhelming results against *T. castaneum* as compared to *R. dominica*. The order of activity of our samples against both the insects were crude saponins > chloroform fraction > ethyl acetate fraction > *n*-hexane fraction > methanolic extract > aqueous fraction.

## Discussions

Our current results revealed that the *T.stocksianum* contains strong anti-pharaoh ant, anti-termite and insecticidal potentials. The insecticidal properties of crude saponins have already been explored [23]. In addition to the insecticidal potential of crude saponins we also confirmed its strong anti-pharaoh ant and anti-termites activities. One of the major source of livelihood for the residents of northern people of Pakistan is the plenty of natural forests [24]. But unfortunately a big loss in these forest can be seen as the soil is a favouring habitat for termites [25-26]. These termites can also destroy wooden materials in homes and other places. Similarly, the pharaoh ants also have the ability to contaminate food stuffs and other goods [3]. To minimize the damage caused by these insects, saponins have already been reported by several researchers to be effective against insects [23]. Different modes of actions of saponins have also been investigated [27-28]. For example, saponins can slow down the passage of food through the gut of insects [29] and also lower the absorption of steroids required for the synthesis of many compounds which are necessary in moulting process of insects. So it might have an inhibitory action on the synthesis of ecdysteroids and hydroecdysone from steroids which are among the most important requirements for moulting process in insects [30]. The inhibitory action of saponins on the digestive proteases of insects have also been studied [31]. Based on the published literature and our current findings, it is obvious that the crude saponins isolated from *T.stocksianum* have strong insecticidal potential. Evaluation of chloroform and ethyl acetate fractions also revealed effective insecticidal properties.

## Conclusion

Our findings conclude that the crude saponins and chloroform fraction may contain such organic compounds that have strong potential against *H. indicola, M. pharaonis, T. castaneum* and *R. dominica.* Furthermore, our study provides a guided platform for the isolation of novel, effective and safe natural insecticidal from *T.stocksianum*.

### *Competing interests*

The authors declare that they have no competing interests.


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### *Author's contributions*

SMMS performed the whole research work under the supervision of AS. SMMS drafted the final version of the manuscript. All the authors have read the manuscript and approved for submission.


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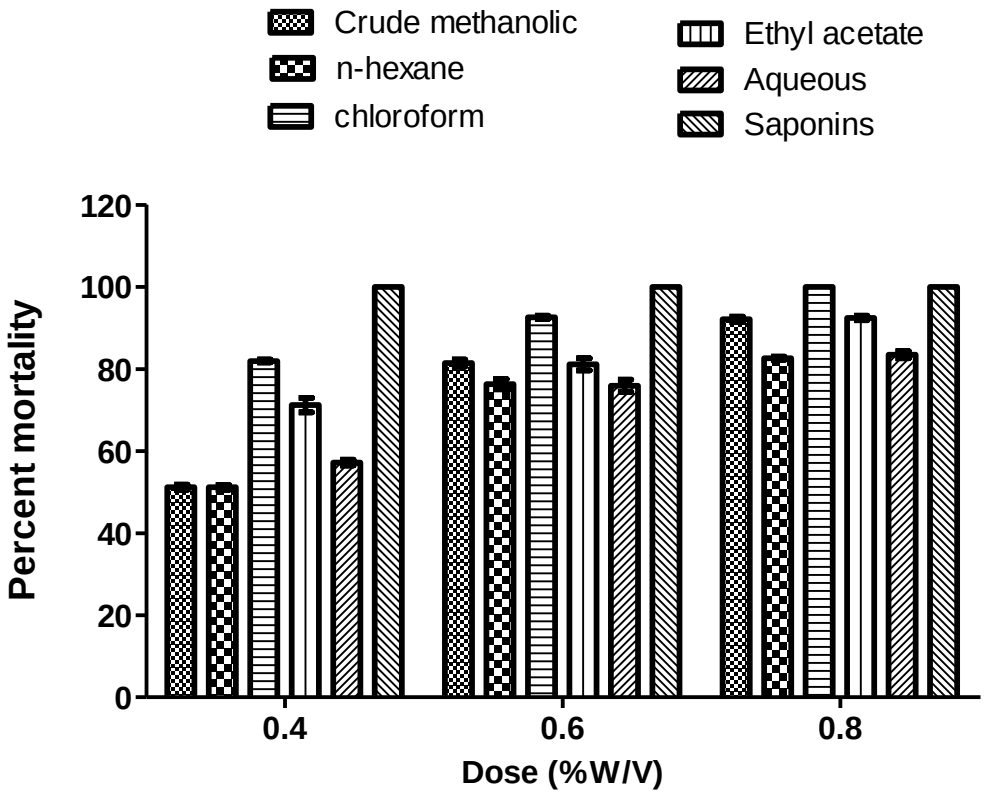
**Figure 1:** Percent anti-termite effect of crude methanolic extract, resultant fractions and saponins of *T. stocksianum.*



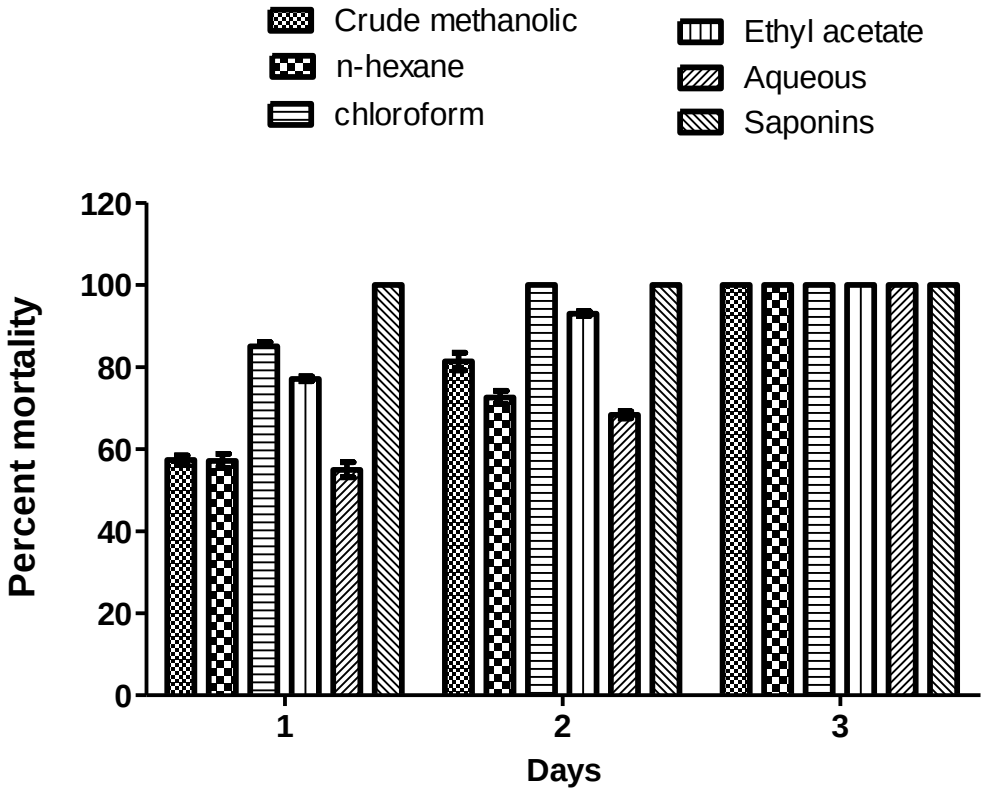

Values were expressed as means of three replicated ± SEM. The data was analyzed by ANOVA. Asterisks show the significant values from control. *$P < 0.05$, **$P < 0.01$.

**Table 1:** $LC_{50}$ Values of crude methanolic extract, resultant fractions and saponins of *T.stocksianum* against Pharaoh ants.

| Samples | Control (DW) | ME | HF | CF | EAF | AF | Saponins |
|---|---|---|---|---|---|---|---|
| $LC_{50}$(% w/v) | -- | 0.36 | 0.4 | 0.01 | 0.01 | 0.26 | 0.01 |

$LC_{50}$ values were determined using Excel sheet. DW; Distilled water.

**Figure 2:** Percent anti-pharaoh ants effect of crude methanolic extract, resultant fractions and saponins of T. stocksianum.




Values were expressed as means of three replicated ± SEM. The data was analyzed by ANOVA. Asterisks show the significant values from control. *P < 0.05, **P < 0.01.

**Table 2:** Insecticidal activity of crude methanolic, various fractions and saponins of *T. stocksianum* against *T. castaneum.*

| Extract | Total number of *T. castaneum* | *T. castaneum* killed (mean±SEM) | Percent mortality (mean±SEM) |
|---|---|---|---|
| Methanolic extract | 20 | 11.67 ± 0.33 | 58.35 ± 1.6 |
| *n*-hexane fraction | 20 | 13.33 ± 0.88 | 66.65 ± 4.4 |
| Chloroform fraction | 20 | 14.67 ± 0.88 | 73.35 ± 4.4 |
| Ethyl acetate fraction | 20 | 13.67 ± 0.67 | 68.35 ± 3.3 |
| Aqueous fraction | 20 | 08.33 ± 0.88 | 41.65 ± 4.4 |
| Saponins | 20 | 16.00 ± 0.58 | 80.00 ± 2.9 |
| Positive control | 20 | 20.00 ± 0.00 | 100.0 ± 0.0 |
| Negative control | 20 | 00 | 00 |

Values are expressed as means of three replicated ± SEM.

**Table 3:** Insecticidal activity of crude methanolic, various fractions and saponins of *T. stocksianum* against *R. dominica.*

| Extract | Total number of *R. dominica* | *R. dominica* killed (mean±SEM) | Percent mortality (mean±SEM) |
|---|---|---|---|
| Methanolic extract | 20 | 10.67 ± 0.88 | 53.35 ± 4.4 |
| *n*-hexane fraction | 20 | 09.67 ± 0.67 | 48.35 ± 3.3 |
| Chloroform fraction | 20 | 13.67 ± 0.33 | 68.35 ± 1.6 |
| Ethyl acetate fraction | 20 | 07.33 ± 0.88 | 36.65 ± 4.4 |
| Aqueous fraction | 20 | 04.00 ± 0.58 | 20.00 ± 2.9 |
| Saponins | 20 | 15.33 ± 0.88 | 76.65 ± 4.4 |
| Positive control | 20 | 20.00 ± 0.00 | 100.0 ± 0.0 |
| Negative control | 20 | 00 | 00 |

Values are expressed as means of three replicated ± SEM.