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PHYTOCHEMICAL SCREENING AND LARVACIDAL ACTIVITY OF LEAF EXTRACT OF SOLANUM XANTHOCARPUM FROM DURG DISTRICT

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In chhattisgarh dengue becomes a primary concern during rainy seasons. Wide range of chemical insecticide has been used which results in the development of resistant within these vectors as well as impair other aquatic lives. The present work includes the use of extract of Solanum xanthocarpum- a widely used medicinal plant as an alternative for chemical insecticide. The data shows the presence of various active phytochemical compounds like alkaloids, terpenoids, tannins, sterols etc. It was observed that larva's mortality rate is proportional to the concentration of the extract furthermore methanolic extract seems to be more effective than aqueous or petroleum ether extract with the mortality percentage of 65% at 50mgL-1 which is quite closer to the value of positive control used (bactevic: 70% in 50mgL-1). Above data suggests that the methanolic extract of Solanum xanthocarpum might be used as an active substitute for chemical insecticide that can not only control these disease causing vectors but also balance the natural bio environment without disturbing the ecosystem.

INTRODUCTION

Mosquitoes act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile Virus infection, etc. Under the Integrated Mosquito Management (IMM), emphasis was given on the application of alternative strategies in mosquito control (Ghosh.A *et al*, 2012). Bacterial and vector borne diseases had been a major issue in Chhattisgarh state during climate change. Sudden change in weather and temperature provide favourable conditions for the growth of pathogens. Dengue has been a leading concern in the state with a total of 2,401 cases in the year 2022. In 2021, a total of 1,086 cases of dengue were found while in 2020, 57 cases were identified across the state (Source-State Epidemic Control of Chhattisgarh). Mosquito control is a vital public health practice throughout the world, especially in the tropics. The most practicable way for reducing incidences of mosquito borne diseases is by controlling mosquito immatures in their breeding sites (Rawani.A *et al*, 2017). Even though chemical vector program has been carried on for long time, these mosquito vectors remain because of repeated use of synthetic products, house hold spray, and insecticides for mosquito control. As a result, the mosquito develops their resistance (Kumar.D and *et al*, 2014). Secondary metabolites serve as a means of defence mechanism of the plants to withstand the continuous selection pressure from herbivore predators and other environmental factors (Ghosh.A *et al*, 2012). Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential

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oils and phenolics from different plants have been reported previously for their insecticidal activities (Ghosh.A *et al*, 2012), (Abubakar AR & Haque M, 2020). Solanum xanthocarpum is a remedial plant which belongs to family solanaceae. It is an important medicinal herb in Ayurveda system of medicines for human health benefits [Chhajed *et al*, 2018]. Medicinal values of this plant are due to the presence of phytochemicals and secondary metabolites. It belongs to one of the family of Dasamula (10 roots) of Ayurveda, and is considered as a noxious weed in India [Poongothai *et al*, 2010]. Previous studies on larvicidal activity of the extracts from different parts of the plant Solanum xanthocarpum against important mosquito vectors in the arid region had been done in which the results shows that the methanol extracts were 2-5 times more effective as compared to the aqueous extracts (Bansal SK *et al*, 2009). However there were only few studies done on larvacidal activity of Solanum xanthocarpum found in rural areas of Durg district. As different region effects the certain properties of plants from the same species, the present work employs hot percolation method for the extraction of plant secondary metabolites from the leaves of Solanum xanthocarpum and their analysis. Comparing these values and investigating their potential against larva of Aedes species which might give further information for the production of chemical free approach for controlling the larva.

II. MATERIAL AND METHODS

Collection & Sample preparation

Leaves of Solanum xanthocarpum were collected from rural localities of Durg district of Chhattisgarh state. Leaves were washed thoroughly under running water and then with distilled water to remove all the dirt and debris from the surface and shade dried, since some plant constituents are photosensitive (Khandelwal.S and Koche.V, 2018). The dried leaves were further incubated at 36 ◦C for 48hours followed by fine powdering by a mixer.

Extraction method

The powdered sample was then subjected to successive cycles of Soxhlet apparatus using 3 solvents i.e. methanol, petroleum ether, aqueous (water); 200 mL each with 20 g of dried sample. The resulting extract was then filtered and concentrated in hot water bath.

Determination of extractive value

The extract was filtered by using Whatmann filter paper no. 1 and filtrate was dried in hot water bath and weighed (Khandelwal.S and Koche.V, 2018). Extractive values in percentage were calculated by using the following formula:

$$\text{Extractive value (\%)} = \frac{\text{weight of extract}}{\text{(weight of plant material)}} \times 100$$

Preliminary Screening Test

Millon's Test for proteins:

5ml of millon's reagent were added to 3ml of sample extract, white precipitate was obtained. After heating precipitate turns brick red.

Molisch's test for Carbohydrates:

The extract was treated with 2-3 drops of 1% alcoholic alpha-napthol and 2ml of sulphuric acid (H2SO4) was added along the side of the test tube, violet ring formed at the junction.

Ferric Chloride Test for Phenols:

A small amount of extract was treated with aqueous 5% ferric chloride. Formation of deep blue or black color indicated the presence of phenols.

Secondary Metabolites Screening Test

Salkowski test for Sterols:

3ml of chloroform (CHCl3) and concentrated sulphuric acid (H2SO4) was mixed and when added with crude sample, red color appears in lower layer indicated the presence of sterols.

Test for Tannins:

Few drops of 1% ferric chloride (FeCl3) were added to the 5ml of aqueous filtrate of the extract. The appearance of brownish green or blue black coloration confirmed the presence of tannins.

Salkowski test for Terpenoids:

0.5 gms of extract was taken and mixed with 2ml of chloroform. 3ml of concentrated sulphuric acid was added. Appearance of reddish brown color at interface indicates the presence of terpenoids.

Test for Cardiac Glycosides:

0.5 ml of extract was dissolved in 2ml glacial acetic acid along with 1-2 drops of 1% ferric chloride (FeCl3). Furthermore 1ml of sulphuric acid (H2SO4) was added to this solution. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

Test for Alkaloids:

4ml Methanol and 400 ml of Glacial acetic acid, along with a few drops of Ammonia was added to the small quantity of dry plant extract. The precipitation indicated the presence of alkaloids. (Husain.N and Kumar.A, 2016).

Test for Flavonoids:

To the aqueous extract, 2.5ml of dilute ammonia was added, followed by few drops of concentrated sulphuric acid (H2SO4). Yellow color appears indicated the presence of flavonoids.

Test for Anthraquinones:

2ml of concentrated sulphuric acid was added to 0.5ml of extract, the solution was then heated. 2.5ml of chloroform was added to the solution and shaken well followed by the addition of the 1ml of dilute ammonia. The change in color indicates the presence of

anthraquinones (Shabeer.M *et al*, 2011).

Test for Saponins (Frothing Test):

0.5 ml of extract was mixed with 5ml of distilled water and shaken vigorously. Appearing of froth indicated the presence of saponins.

Collection of mosquito larva & their Identification

Mosquito larvae were collected from the stagnant water area around Durg district with the help of the net and were transferred into the paper cups and transported carefully to the department of biotechnology. They were then identified as the larvae of Aedes aegypti under a microscope with the help of an expert on the basis of their morphological key features.

Larvacidal Assay

The assay was developed according to the methodology of (Wandscheer *et al*, 2004) & (Cruz-Estrada *et al*, 2013). Three concentrations of Solanum xanthocarpum extract were prepared by diluting 1 mL of the initial extracts (1, 2, and 5mgmL-1) in 100 mL of water, obtaining final doses of 10 mg L-1, 20 mg L-1, and 50 mg L-1; dose range was selected based on (Wandscheer *et al*, 2004). Ten larvae were placed in each polyethylene plastic containers with test solutions (100 mL) at a temperature from 25 to 30 °C and 12 h photoperiod. The tests were performed three times (Manzano.P *et al*, 2020). Negative control (NC) was water, and positive control (PC) was Bactivec.

RESULT & DISCUSSION

Table: 1 Extractive values of air dried plant extract from various solvents.

Table: 1Extractive values of air dried plant extract from various solvents.

Solvents	Color & Consistency	Extractive Value (in %)
Methanol	Dark Green, Sticky	6.0%
Aqueous	Greenish Brown, Viscous	5.5%
Petroleum Ether	Light Green, Sticky	4.0%

Table: 2 Preliminary Screening of Extract of *Solanum xanthocarpum* in different solvents.

Test	Aqueous	Methanol	Petroleum Ether
Protein	+	+	+
Carbohydrate	+	+	-
Phenol	+	+	+

Table: 3 Phytochemical Screening of Extract of *Solanum xanthocarpum* in different solvents.

Test	Aqueous	Methanol	Petroleum Ether
Sterols	+	+	+
Tannins	+	+	-
Terpenoids	-	-	+
Cardiac Glycosides	+	+	+
Alkaloids	+	+	+
Flavonoids	+	+	-
Anthraquinones	+	+	+
Saponins	+	+	-

Graph: 1 Shows larvacidal activity of various extract against the larva of *Aedes aegypti*

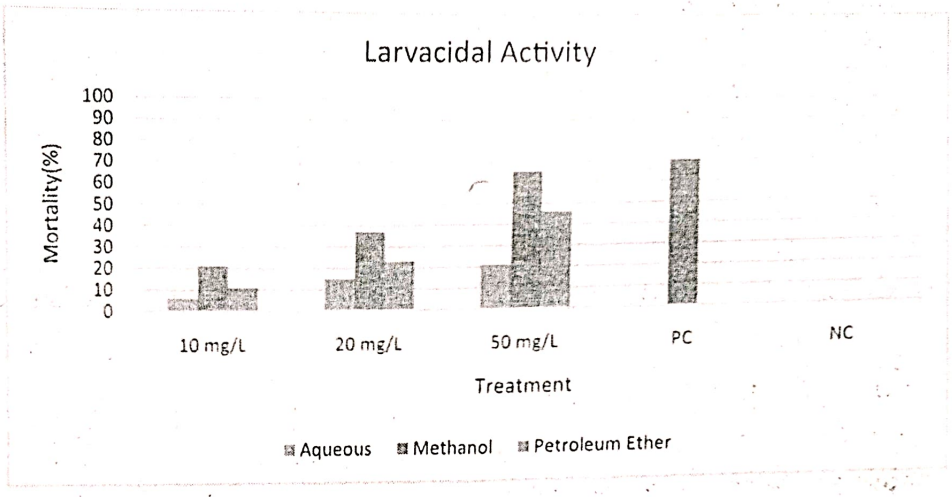
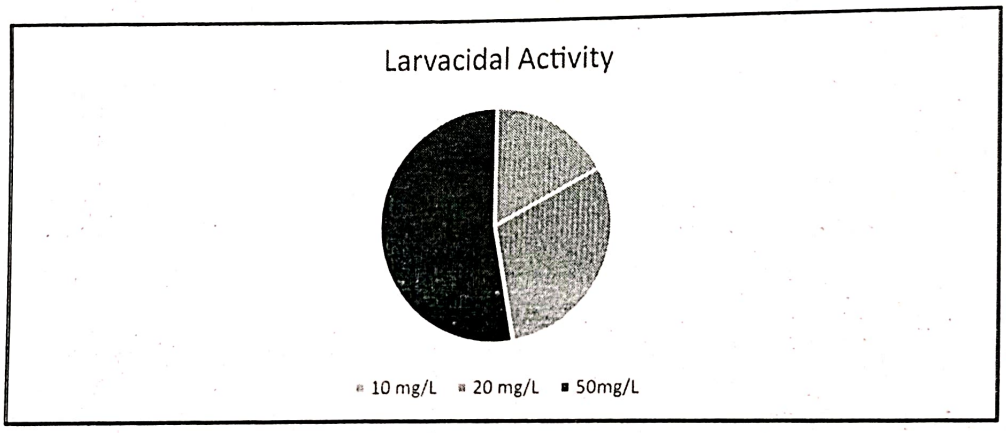


Fig: 1 Pie shows the larvacidal activity of Methanol extract at different concentration



The above data shows that the extractive value of methanolic extract is somewhat greater than that of aqueous and petroleum ether (table1). Preliminary tests confirmed the presence of protein, carbohydrate and phenols in the plant extract whereas carbohydrate was seen absent in petroleum ether extract (table2). Furthermore phytochemical screening of secondary metabolites showed the presence of some active compounds like alkaloids, sterols, anthraquinones, glycosides and terpenoids however tannins, saponins and flavonoids are found absent in petroleum ether extract (table3). Larvacidal activity of Solanum xanthocarpum showed maximum result in methanolic extract as compared to petroleum ether and aqueous (graph1). The graph insinuates that mortality rate (%) depends upon the concentration of the extract as the concentration was increased the mortality rate gradually increases in case with each solvent (fig1). Also high mortality in case of methanolic extract which is close to the value of positive control suggests that it can be used as promising alternative of pesticides against mosquito larva. The above data might also be useful in near future to investigate the reason for elevated larvacidal activity of methanolic extract of Solanum xanthocarpum.

CONCLUSION

Solanum xanthocarpum belongs to the family solanaceae one of the alkaloid-rich

family. It has been used for its multiple medicinal values which provide cures for various diseases. The above work alludes that because of presence of active phytochemical compounds like alkaloids, terpenoids, the plant extract shows larvacidal activity with high mortality rate in case of its methanolic extract as compared to aqueous and petroleum ether. The mortality also depends upon the concentration of extract used. The data gives relation between mortality rate and concentration of the extract used, moreover it suggests the high larvacidal potential of methanolic extract of Solanum xanthocarpum might be useful for preparing chemical free pesticides in future.

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