

Estimation of Phytochemical Components from Cassia Tora and To Study Its Larvicidal Activity.

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ABSTRACT:- Diseases due to Mosquito bites are a common health problem all over the world. The use of medicinal plant continues to play an important role in the management of different disease. Effective, safe & cheap medicinal plants may appear as a good alternative potential source for controlling microbial infection. Cassia tora is one of the medicinal plants having many useful properties. Seed extract with different concentration of 0.1%, 0.2%, 0.3%, 0.4% alcoholic extract were used to study the larvicidal activity of Cassia Tora (leguminaceae) seeds extract (powder) against the larvae of anopheles stephensi. The extract exhibited larvicidal activity of varied efficacy with different concentrations. 0.4 % cassia tora extract gave 80% mortality in the larvae of AnophelesStephensi. Besides this, some phytochemical studies were also carried out which showed the presence of saponin, glycosides, protein, tannin and carbohydrates.

KEY WORDS: mosquito larvae, cassiatora seeds. Phytochemical content

I. INTRODUCTION

According to the latest WHO estimates, released in December 2014, there were about 198 million cases of malaria in 2013 (with an uncertainty range of 124 million to 283 million) and an estimated 584 000 deaths (with an uncertainty range of 367 000 to 755 000). Most deaths occur among children living in Africa where a child dies every minute from malaria. Malaria is caused by *Plasmodium* parasites. The parasites are spread to people through the bites of infected *Anopheles* mosquitoes, called "malaria vectors", which bite mainly between dusk and dawn. There are four parasite species that cause malaria in humans:

- *Plasmodium falciparum*
- *Plasmodium vivax*
- *Plasmodium malariae*
- *Plasmodium ovale*.

Plasmodium falciparum and *Plasmodium vivax* are the most common. *Plasmodium falciparum* is the most deadly. The *Anopheles* mosquitoes are vectors for all the four species of the malarial parasite.

In recent years, some human cases of malaria have also occurred with *Plasmodium knowlesi* – a species that causes malaria among monkeys and occurs in certain forested areas of South-East Asia.

Malaria is transmitted exclusively through the bites of *Anopheles* mosquitoes. The intensity of transmission depends on factors related to the parasite, the vector, the human host, and the environment.

About 20 different *Anopheles* species are locally important around the world. All of the important vector species bite at night. *Anopheles* mosquitoes breed in water and each species has its own breeding preference; for example some prefer shallow collections of fresh water, such as puddles, rice fields, and hoof prints. Transmission is more intense in places where the mosquito lifespan is longer (so that the parasite has time to complete its development inside the mosquito) and where it prefers to bite humans rather than other animals. For example, the long lifespan and strong human-biting habit of the African vector species is the main reason why about 90% of the world's malaria deaths are in Africa.

Transmission also depends on climatic conditions that may affect the number and survival of mosquitoes, such as rainfall patterns, temperature and humidity. In many places, transmission is seasonal, with the peak during and just after the rainy season. Malaria epidemics can occur when climate and other conditions suddenly favour transmission in areas where people have little or no immunity to malaria. They can also occur when people with low immunity move into areas with intense malaria transmission, for instance to find work, or as refugees.

Human immunity is another important factor, especially among adults in areas of moderate or intense transmission conditions. Partial immunity is developed over years of exposure, and while it never provides complete protection, it does reduce the risk that malaria infection will cause severe disease. For this reason, most malaria deaths in Africa occur in young children, whereas in areas with less transmission and low immunity, all age groups are at risk. Resistance to antimalarial medicines is a recurring problem. Resistance of *P. falciparum* to previous generations of medicines, such as chloroquine and sulfadoxine-pyrimethamine (SP), became widespread in the 1970s and 1980s, undermining malaria control efforts and reversing gains in child survival.

In recent years, parasite resistance to artemisinins has been detected in 5 countries of the Greater Mekong subregion: Cambodia, Laos, Myanmar, Thailand and Viet Nam. While there are likely many factors that contribute to the emergence and spread of resistance, the use of oral artemisinins alone, as monotherapy, is thought to be an important driver. When treated with an oral artemisinin-based monotherapy, patients may discontinue treatment prematurely following the rapid disappearance of malaria symptoms. This results in incomplete treatment, and such patients still have persistent parasites in their blood. Without a second drug given as part of a combination (as is provided with an ACT), these resistant parasites survive and can be passed on to a mosquito and then another person. If resistance to artemisinins develops and spreads to other large geographical areas, the public health consequences could be dire. WHO recommends the routine monitoring of antimalarial drug resistance, and supports countries to strengthen their efforts in this important area of work. More comprehensive recommendations are available in the "WHO Global Plan for Artemisinin Resistance Containment (GPARC)", which was released in 2011. For countries in the Greater Mekong subregion, WHO has issued a regional framework for action titled "Emergency response to artemisinin resistance in the Greater Mekong subregion" in 2013.

Vector control is the main way to reduce malaria transmission at the community level. It is the only intervention that can reduce malaria transmission from very high levels to close to zero. Use of Medicinal plants plays an important role in the prevention due their larvicidal properties. They have high efficacy against resistant species as well. Cassia tora is one such plant whose larvicidal activity on mosquitoes from the Anopheles family is yet to be researched.

Cassia tora (Leguminosae) is a wild crop and grows in most parts of India as a weed. According to Ayurveda, the leaves and seeds are acrid, laxative, anthelmintic, ophthalmic, liver tonic, cardio tonic and expectorant (Ahmed et al. 1998). The leaves and seeds are also useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, and cardiac disorders (Chan & Peria in 2001). The known chemical components of Cassia tora are Anthraquinones, chrysophanol, Emodin, obtusifolin, obtusin, chryso-obtusin, auranto-obtusin, and their glycosides. Naphopyrones, rubrofusarin, norubrofusarin, rubrofusarin, etiobioside, Toralactone, torachryson. Its root contains 1, 3, 5-trihydroxy-6,7-dimethoxy-2-methylanthraquinone and beta-sitosterol while the seeds contain napho-alpha-pyrone, toralactone, chrysophanol, physcion, emodin, rubrofusarin, chrysophonic acid-9-anthrone. Emodin, tricostatin, stigmasterol, beta-sitosterol, beta-D-glucoside, ferindlen, palmitic, stearic, succinic and tartaric acid, uridine, quercetin and isoquercetin have been isolated from leaves (Davis 1994 & Desta, 1993). Antibacterial, antiplatelet aggregation, hepatoprotective, campophosphodiesterase inhibitory activity, antifungal, anti-inflammatory and antiestrogenic, hypolipidemic, antimutagenic, and antioxidant activities have been evaluated (Devi et al. 1994, Duke & Beckstrom, 2002 & Karaman et al. 2003) previously.

Literature survey revealed that the plant extract has yet not been screened for its traditional claim of larvicidal activity. Therefore the objective of this work was to estimate the phytochemical components & to explore the larvicidal activities of Cassia tora seeds on Anopheles stephensi.

Taxonomical classification

Kingdom	Plantae
Division	Rosopsida
Order	Fabales
Family	Fabaceae (Leguminosae)
Sub. Family	Caesalpinioideae
Genus	<i>Cassia</i>
Species	<i>Torra</i>

Physical Characteristics of Cassia tora seeds

Col;out of seeds	Light Brown(like coffee)
Size	0.3-0.4 cm long
Taste	Bitter salty. Slightly cold in nature
Odor	Odorless or sometimes slightly bitter odor

Cassia tora L., (Cassia obtusifolia L.), Caesalpinaceae, occurs throughout India as a weed.

II. MATERIALS AND METHODS

Collection of seeds:

Seeds of Cassia tora were collected from the area of Badnera railway station. Dist. - Amravati (Maharashtra) in month of December. The plant was originally authenticated by Dr.Z.H.Khan, H.O.D.of Biochemistry, and Shri Shivaji College of arts, com, and sci. Akola. Dist. - Akola.

Extraction of Cassia tora:

Seeds were washed with tap water; shade dried, powdered in a kitchen blender and were stored in an air tight plastic bag. Then powdered seeds passed through sieve # 10 was defatted by the whatman filter paper and introduced into the soxhlet apparatus using petroleum ether (60-80) as a solvent. After complete defatting, powder was air dried for removing trace of the petroleum ether then packed in whatman filter paper and introduced into the soxhlet apparatus and extract with benzene as a solvent for complete extraction. The extract was filtered, concentrated and dried in water bath. Dried extract was transferred into air tight bottles and the percentage yield was calculated and stored at cool place.

The initial weight of the powder before using soxhlet method was: - 31.11 gm.

After defatting by petroleum ether (for 3 hours) wt. of the powder:-29.44 gm.

After defatting by benzene (for 2 hours) wt. of the powder: - 27.62 gm.

Phytochemical evaluation:

1. Glycosides (Modified Brontrager's test):-

Test procedure

Extract was hydrolyzed with dilHCl, and then subjected to test for glycosides.

Extract was treated with ferric chloride solution & immersed in boiling water for about 5 min. the mixture was cooled and shaken with an equal volume of benzene.

The benzene layer were separated and treated with ammonia solution, formation of rose pink colour in the ammonical layer indicates the anthraquinone glycosides.

2. Saponin (forth test):

3. Fixed oils & fats. (Stain test):

Test procedure:-

Small quantity of extract was pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oil.

4. Protein (By Folin Lowry method):

5. Determination of Total Carbohydrate by Anthrone Method

Materials:

- 2.5 N HCl
- *Anthrone reagent:* Dissolve 200 mg anthrone in 100 mL of ice-cold 95% H₂SO₄. Prepare fresh before use.
- *Standard glucose:* Stock—Dissolve 100 mg in 100 mL water. Working standard—10 mL of stock diluted to 100 mL with distilled water. Store refrigerated after adding a few drops of toluene
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Estimation of tannin from cassia tora

- **Reagents:-**

- 1)folin-dennis reagent:- 750 ml distilled water + 100 gm sodium tungstate + 20 gm phosphomolybdic acid + 50 ml 85% phosphoric acid, heat under reflux for 2 hours. And cool and make up the volume 1 liter.
- 2) Saturated sodium carbonate: - use the deconted clear solution.
- 3) 10% homogenate.
- 4) Tannic acid solution:- 100 mg. of tannic acid per 100 ml distilled water, use as working std.

- **Observation:-**

- Cassia tora powder = 0.6 gm / 0.1 ml = 6 mg / gm.

Larvicidal activity:-

Mosquitoes are the major vectors for many diseases such as dengue fever, yellow fever, malaria, filariasis, Japanese encephalitis, and other fevers (Service 1983). Mosquito-borne diseases cause a high level of morbidity and mortality, but they also are responsible for great socioeconomic loss. In 2002, WHO reported that 273 million people.

Indeed, the present recrudescence of these diseases is due to the higher number of breeding places in today's throwaway society, the increasing resistance of mosquitoes to current commercial insecticides (Cicciet al. 2000), and toxicity in the public use (Zadikoff 1979). These problems indicate a need for new and improved larvicides and strategies for protection from mosquito attack. Researchers are now looking for natural larvicides that do not have ill effects on nontarget populations and are easily degradable. The search is underway to find newer larvicides that will be effective and safe, and also readily available at a low cost.

In recent years, the medicinal plants have received much attention as potentially useful bioactive compounds against early 4th-stage mosquito larvae (Kim et al. 2001b). Therefore, **“the aim of the present study is to assess the larvicidal activity of medicinal plants against *Anopheles stephensi*.”**

Procedure of larvicidal activity:

The larvae's of *Anopheles* mosquito were collected from the lotus tank of water. From Badnera, Dist-Amravati, India. The larvae were isolated from the tank with the help of mug and sieve and maintained in the distilled water. Fresh larvae were taken each time..

Sample preparation:

The seeds of cassia tora were collected during the month of December 2011. The powder of seeds was made with the help of mixer grinder. Then the extract were formed as per given in the above procedure (extraction of cassia tora).

Bioassay:

Concentration of the test sample extract was extracted by petroleum ether (60-80). Then it is again defatted with Benzene. Then after the dried defatted powder of seeds were used to detect the larvicidal activity of species *Anopheles stephensi*.

The extract was suspended in the distilled water in the form of particular percentage. Groups of 10 larvae's *Anopheles stephensi* were put into the every Petri plate. In the following manner,

0.1% - 0.2 gm of extract in 20 ml distilled water.

0.2% - 0.4 gm of extract in 20 ml distilled water.

0.3% - 0.6 gm of extract in 20 ml distilled water.

0.4% - 0.8 gm of extract in 20 ml distilled water.

Std - 1 gm of extract in 20 ml distilled water.

These all plates were kept for 24 hours. Control larvae were held at same condition describe earlier. Larvicidal activity was evaluated 24 hours after treatment. All treatment was replicate 4 times. No mortality was obtained in each control. Treatment means were compared and where differences occurred.

III. RESULT

The initial weight of the *Cassia tora* powder before using soxhlet apparatus was 31.11 gm. After defatting by petroleum ether for 3 hrs. weight of powder 29.44 gm. While defatting by benzene for 2 Hrs. Gave 27.62 gm. A proposed work confirms the presence of various phytochemical like glycosidase, saponin, fixed oils & fats. Besides these it confirms the presence of Protein, Carbohydrates & Tannin estimated were 0.7 mg/ml, 4.8 to 7.7 mg/ml respectively. After 24 hrs. Exposure, the extract of *Cassia tora* revealed various larvicidal activity according to tested concentration. At concentration of standard level *Cassia tora* seeds caused 100% mortality, the concentration of 0.3% *Cassia tora* seeds shows 60% mortality, while the concentration of 0.2% the *Cassia tora* seeds gave 40% mortality. At the concentration of 0.1% the *Cassia tora* seeds caused 30% mortality against *Anopheles Stephensi*.

1. Glycosides (Modified Brontrager's test):-

Extract was treated with ferric chloride solution & immersed in boiling water for about 5 min. the mixture was cooled and shaken with an equal volume of benzene. The benzene layer were separated and treated with ammonia solution, formation of rose pink colour in the ammonical layer indicates the anthraquinone glycosides.

2. Saponin (foam test):

Test procedure Extract was dil in distilled water to 20 ml. & this was shaken in a graduated cylinder for 15 min. formation of 1 cm. layer of foam indicates the presence of saponin.

3. Fixed oils & fats. (Stain test):

Small quantity of extract was pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oil.

4. Protein (By Folin Lowry method):

Estimation of protein by Folin Lowry method.

Result: the concentration of protein in cassia tora extract found to be 0.7 mg/ml.

5. Determination of Total Carbohydrate by Anthrone Method

Result: The concentration of carbohydrate in cassia tora extract found to be 4.6 mg. and 7.4 mg.

6. Estimation of tannin from Cassia tora:-

Result:-

The amount of tannic acid in the cassia tora seeds was found to be as follows,

Cassia tora powder = 6 mg / gm.

IV. DISCUSSION

The plant cassia tora medicinal plant belonging to the family leguminaceae shows strong larvicidal activity against *Anopheles stephensi*. The present work reveals the larvicidal activity in extract obtained from cassia tora plant. The alcoholic extract shows the strong larvicidal activity against *Anopheles stephensi*. The seeds of medicinal plant Cassia tora contain glycosides, flavonoids, saponin, alkaloid, tannin, protein, carbohydrates, gum and phenolic compound but maximum activity were shown by alcoholic extract against *Anopheles stephensi*.

Many plant extracts and phytochemicals possess larvicidal activity against various mosquito species (Berenbaum 1989, Sukumar et al. 1991). Jang et al. (2002) reported that the most promising botanical mosquito larvicides are Graminales, Leguminosae, Polygonaceae, Labiatae, Magnoliaceae, and Pedalidaceae, whereas *Amphicarpaeaedgeworthii*, *Cassia obtusifolia*, *Cassia tora*, *Glycine max* var. *woolalikong*, *Lathyrus japonica*, *Panicum miliaceum*, *Rhynchosia volubilis*, *Schizandranigra*, and *Viciatetraspermacan* can be employed as economical and environmentally friendly mosquito larvicides. Various compounds including phenolics, terpenoids, and alkaloids exist in plants (Swain 1977, Wink 1993, Kim et al. 2001b). These compounds may jointly or independently contribute to generation of larvicidal activities against mosquitoes (Assabgui et al. 1997, Hostettmann and Potterat 1997).

These results suggest that chemical composition of the cassia tora extract from the plant species may be different. Our work suggest that it is useful for managing mosquito larvae. Further works on these plant-derived active constituents against mosquito larvae is needed for developing them into effective formulations for control of mosquito larvae. Furthermore, further research to identify the biologically active substances in the plant *cassia tora*, which showed the most potent larvicidal activity, is yet in progress.

V. CONCLUSION

This study thus conclude that, *Cassia tora* is a medicinal plant showing larvicidal activity against *Anopheles stephensi* which was confirmed by its alcoholic extract against *Anopheles stephensi*.

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