

## PHYTOCHEMICAL COMPOSITION AND LARVICIDAL EFFICACY OF CALENDULA OFFICINALIS AND AZADIRACHTA INDICA

R. Manohari<sup>1</sup>, R. Sujapandian<sup>2\*</sup>, J. Jayachitra<sup>3</sup> and V. Bharathi<sup>4</sup>

<sup>1</sup>Post Graduate Research Department of Biochemistry, Rabiammal Ahamed Maideen College for Women – 610001, Tamilnadu, India.

<sup>2</sup>Assistant Professor, Post Graduate Research Department of Biochemistry, Rabiammal Ahamed Maideen College for Women – 610001, Tamilnadu, India.

<sup>3</sup>Assistant Professor, Post Graduate Research Department of Biochemistry, Rabiammal Ahamed Maideen College for Women – 610001, Tamilnadu, India.

<sup>4</sup>Assistant Professor, Department of Biochemistry and Shrimati Indira Gandhi College, Tiruchirappalli, Tamil Nadu.

Article Received on  
03 June 2018,

Revised on 23 June 2018,  
Accepted on 14 July 2018,

DOI: 10.20959/wjpr201815-12849

### \*Corresponding Author

**R. Sujapandian**

Assistant Professor, Post  
Graduate Research  
Department of  
Biochemistry, Rabiammal  
Ahamed Maideen College  
for Women – 610001,  
Tamilnadu, India.

### ABSTRACT

Mosquitoes are the major public health problem throughout the world. Among the 3492 Species of mosquitoes recorded worldwide, more than a hundred species are capable of transmitting various diseases in human and other vertebrates. One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides of biological origin as a simple and sustainable method of mosquito control. The effects of botanical derivatives against mosquito have been reviewed. In the present study, to identify alternative natural eco-friendly larvicidal agent using *Calendula officinalis* and *Azadirachta indica* formulation.

**KEYWORDS:** Biodiversity, larvicidal, *Calendula officinalis* and

*Azadirachta indica* etc.

### INTRODUCTION

Many approaches have been developed to control mosquito menace. One such approach to prevent mosquito borne disease is by killing mosquito at larval stage. The current mosquito control approach is based on synthetic insecticides. Even though they are effective, they

created several struggles like insecticide resistance, pollution; toxic side effect on human begins. One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides of biological origin as a simple and sustainable method of mosquito control. The effects of botanical derivatives against mosquito have been reviewed.<sup>[1]</sup>

*Azadirachta indica* (Meliaceae) commonly known as neem is native of India and naturalized in most of tropical and subtropical countries is of great medicinal value and distributed wide spread in the world. The Chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, flavonoids, triterpenoids, phenolic compounds, Carotenoids, steroids and ketones. Azadirachtin is actually a mixture of seven isomeric compounds labeled as azadirachtinA-G and azadirachtin E is more effective.<sup>[2]</sup>

Other compounds that have a biological activity are salannin, volatile oils, meliantriol and nimbin.<sup>[3]</sup> Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases.<sup>[4]</sup> The plant extracts have been developed and proposed for use as antimicrobial substances.<sup>[5]</sup> Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine<sup>[6]</sup> Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential.<sup>[7]</sup>

Medicinal plants are used from ancient times in treatment of various human & animal disorder. Medicinal and aromatic plants are the most widely used form of medicine in the world today where medicinal and aromatic plants contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds.<sup>[8]</sup> Natural products are gaining a revitalized attention in medical community and their therapeutic uses are gradually increasing. As many synthetic drugs have revealed serious side effects. Therefore, a better strategy is to look for natural substances with strong pharmacological action and less cytotoxicity.

In the last few years much attention was directed to the potential health promoting properties of phenolic phytochemicals. Search for safe herbal remedies with potent anti-inflammatory and antipyretic received momentum recently as the available paracetamol and aspirin have toxic effect to various organ of the body. *Calendula* was cultivated by the Egyptians, Greeks, Hindus and Arabs, *Calendula* grew in European gardens and has been used medicinally since

the 12th century.<sup>[9]</sup> The name *Calendula* is from the Middle English *calends* derived from Latin *kalendae*, which means the day of the new moon.<sup>[10]</sup> The flowers are used in diverse preparations, mainly ointments for the treatment of diverse dermatological conditions such as wounds, ulcers, eczema, burns, bruises, eruptions, varicoses and haemorrhoids.<sup>[11]</sup> Many other properties have been attributed to the flower preparations such as choleric, anti-inflammatory, analgesic, anti-cancer, bactericidal, diuretic and tonic actions.<sup>[12]</sup>

## MATERIALS AND METHOD

### Extraction of Plant Material

Aqueous, chloroform and alcoholic extracts were prepared according to the methodology of Indian Pharmacopoeia. The shady dried plants materials were subjected to pulverization to get coarse powder. The coarse powder material was subjected to soxhlet extraction separately and successively with alcohol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50°C). The aqueous and alcohol extracts put in air tight containers stored in a refrigerator.

### PHYTOCHEMICAL SCREENING

The *Calendula officinalis* and *Azadirachta indica* formulation was tested for steroids, alkaloids, sugar, phenolic compounds, flavonoids, saponins, tannins, anthraquinone and amino acids. Phytochemical screening of the extract was carried out according to the standard method.

### COLLECTION OF MOSQUITO LARVAE SAMPLE

*Anopheles* larvae were collected from sewage of Marudhandakurichi village in Trichy, Tamil Nadu were transported to the laboratory in plastic containers. In the laboratory, the larvae were transferred to enamel larval trays until adult emergence. After emergence, the adult mosquitoes were identified upto genus level and confirmed before rearing. Mean room temperature of 27 ±2 °C and a relative humidity of 70- 80% were maintained in the insectary. The larvae were fed with larval food (fish feed) for increasing their population.

### LARVICIDAL BIOASSAY

WHO protocol with minor modifications was adopted for the study. The tests were conducted in plastic containers. Third instar larvae were obtained from laboratory. The stock solution (20ml) were prepared by inoculate 2g of *Calendula Officinalis* and *Azadirachta Indica* formulation (alcohol extract) sample into the distilled water. Ten healthy larvae were released

into each 250 ml glass beaker containing 100 ml of water and test concentration. From the stock solution, concentrations of 1000, 2000, 3000, 4000 and 5000 ppm were added. Mortality was observed for 24 and 48 hours after treatment. The larvae were considered dead when they showed no sign of movement when they were probed using a needle.

### PREPARATION OF REPELLENT

The biological repellent has been prepared by using *Calendula Officinalis* and *Azadirachta Indica* formulation. 0.5g of alcohol extract of *Calendula Officinalis* and *Azadirachta Indica* formulation was added with 5ml of 95% alcohol and then the mixture is dissolved using a 75% alcohol respectively. The solution was packed in the empty good night container. For positive control the commercially available good night liquid mosquito repellent was used.

### IN VIVO ASSAY OF REPELLENT ACTIVITY

A closed wooden chamber was selected and a small hole was made at one side in order to insert an extension wire on it. Inside the wooden box the glass beaker containing mosquito was placed and the good knight electric mosquito repellent was plugged in the extension box. This setup was observed until it gets eradicated. On next day, the same setup was carried out using biologically prepared *hebal* repellent and were observed.

### RESULT AND DISCUSSION

**Table 1: Phytochemical Screening Of *Calendula Officinalis* And *Azadirachta Indica* Formulation.**

S. No.	Phytochemical	water	alcohol
1	Alkaloids	+	+
2	Flavanoids	+	+
3	Steroids	-	-
4	Tannins	+	+
5	Terpanoids	-	-
6	Quinine	-	+
7	Coumarins	+	+
8	Starch	-	-
9	Saponins	+	+
10	Phenols	-	+

### PHYTOCHEMICAL SCREENING

The preliminary phytochemical analysis was carried out in the extracts of *Calendula Officinalis* and *Azadirachta Indica* formulation. The phytochemical analysis was carried out in the two different extracts. The qualitative analysis of the ethanolic and water extracts of

*Calendula Officinalis* and *Azadirachta Indica* formulation revealed the presence of alkaloid, flavanoid, terpenoid, saponin, steroid, tannin and phenolic compounds, whereas starch and volatile oil were absent. The ethanolic extract of *Calendula Officinalis* and *Azadirachta Indica* formulations showed on indication of the presence of saponin, coumarin, flavonoids, tannin, phenolic compound, and quinone were confirmed in suitable chemical test. The aqueous extract of *Calendula Officinalis* and *Azadirachta Indica* formulation contain alkaloid, terpenoid, tannin, saponin and phenolic compound. Moreover, the highest yield was also observed in ethanolic extract and hence this was selected for further studies (Table 1) (Plate 2).

Ethanol proved to be the best solvent as it contained most of the phytochemical tested while acetone, benzene, petroleum ether and methylene chloride had only four of the phytochemicals tested.<sup>[13]</sup>

**Table 5: Larvicidal Bioassay.**

S. No.	Concentration(ppm)	Mortality Rate(24h) (100%)	Mortality Rate(48h) (100%)
1.	1000ppm	30%	40%
2.	2000ppm	30%	50%
3.	3000ppm	30%	60%
4.	4000ppm	40%	60%
5.	5000ppm	60%	70%

These obtained finding correlates with that obtained by<sup>[14]</sup> who reported that ethanolic extract of *Calendula officinalis* Linn. Flower contains flavonoids. specified that flavonoids present in *Calendula officinalis* Flower are isorhamnetin-3-O-hesperidoside, quercetin.<sup>[15]</sup> suggested that these ingredients are responsible for *Calendula officinalis* antioxidant activities and<sup>[16]</sup> added that flavonoids has also antinociceptive and anti-inflammatory effect.<sup>[17]</sup> added that *Calendula officinalis* flower ethanolic extract contains other flavonoids as isoquercetin, isorhamnetin-3-O-D-glycoside, narcissin, calendoflaside, calendoflavoside, calendoflavobioside, rutin, isoquercitrin, neohesperidoside, isorhamnetin-3-O-2Grhamnosyl rutinoid, isorhamnetin-3-O-rutinoid, quercetin-3-O-glucoside and quercetin-3-O-rutinoid. *Calendula officinalis* methanolic extract contains also some of these flavonoids as Quercetin-3-o-glucoside, Rutin, and Isohamnetin-3-o-glucoside and also gallic acid as mentioned by<sup>[18]</sup> who added that these ingredients are present also in aqueous extract.<sup>[14]</sup> stated that *Calendula officinalis* ethanolic, aqueous and methanolic extract contains also Glycosides and Saponin.<sup>[20]</sup> confirmed presence of these chemical compounds in methanolic

extract.<sup>[21]</sup> reported that phytochemical analysis of *Calendula officinalis* Linn. Aerial parts aqueous extract contains also flavonoids and tannins. but he diversely mentioned that it does not contain saponin and contains alkaloids.

There are about 45,000 plant species in India with capacity to produce a large number of organic chemicals concentrated hotspot in the region of Eastern Himalayas, of high structural diversity.<sup>[22]</sup> The result of phytochemicals in the present investigation showed that the plant leaves contain components like tannins, glycosides, reducing sugar and triterpenes. This study reports the presence of different phytochemicals with biological activity that can be valuable therapeutic index.<sup>[23]</sup> In the present study, we have found that the biologically active phytochemicals were present in the methanolic extracts of few medicinal plants.

### LARVICIDAL BIOASSAY

In the current investigation, larvicidal activity of *Calendula Officinalis* and *Azadirachta Indica* formulation alcohol extract was carried out against *Anopheles* species. Mortality was observed for 24 and 48 hours. The results of the present study revealed that, alcohol extract of *Calendula Officinalis* and *Azadirachta Indica* formulation possessed high larvicidal activity. The extracts showed moderate larvicidal effects after 24h of exposure at 1000ppm. However, the highest larval mortality was found at 5000ppm after 48 h (Table 5) (Plate 4).

Plant extract against fourth instar larvae of *Ae. aegypti*, *An. Stephensi* and *Cx. Quinquefasciatus* after 24 hrs exposure. The results clearly indicate that the plant extract of *L. Camara aculeate* at very low concentrations was toxic against all the three mosquito species tested. The methanolic plant extract was found to be more potent against *Cx. Quinquefasciatus* and *An. Stephensi* with LC<sub>50</sub> and LC<sub>90</sub> values of 35.36 ppm and 107.42 ppm and 35.65 ppm and 106.95 ppm when compared to *Ae. Aegypti* with LC<sub>50</sub> and LC<sub>90</sub> of 39.54 ppm and 118.62 ppm respectively. Methanolic extract of *L. Camara aculeate* showed 100% mortality at 150 ppm against the fourth instar larvae of *An. Stephensi*, *Ae. aegypti* and *Cx. Quinquefasciatus*. Ethanolic whole plant extracts were found to be equally effective against the fourth instar larvae of all the three mosquito species. LC<sub>50</sub> and LC<sub>90</sub> values were 50.17 ppm and 155.64 ppm against *Cx. Quinquefasciatus* when compared to *Ae. Aegypti* (60.93 ppm and 181.99 ppm) and *An. Stephensi* (79.03 ppm and 237.19 ppm) respectively. All other tested extracts also showed mosquito larvicidal activity at a relatively high concentration when compared to methanol and ethanol plant extracts.<sup>[24]</sup>

## CONCLUSION

Alcohol and aqueous extracts of *Calendula Officinalis* and *Azadirachta Indica* formulation were prepared based on standard protocols and they were assayed for their following test.

Preliminary phytochemical analysis of aqueous extract and alcohol extract of *Calendula Officinalis* and *Azadirachta Indica* formulation showed positive results for terpenoids, flavonoids, alkaloid, phenol, saponin, tannins, coumarins whereas, quinone was positive only on aqueous extract.

For larvicidal bioassay, WHO standard procedure was followed with slight modifications and observed after 24 hours and 48 hours. At the end of 24 hours according to extract concentration the mortality rate, at 1000ppm, 2000ppm and 3000ppm concentration only 30% of mortality was observed. In 4000ppm concentration 40% of mortality rate was observed. In 5000ppm concentration 60% of mortality was observed, whereas after 48 hours all the larvae were died irrespective of concentrations. Based on the present study, the alcohol extract of *Calendula Officinalis* and *Azadirachta Indica* formulation could be used as a best larvicidal drug candidate.

## ACKNOWLEDGEMENT

The authors are grateful thank to the Secretary, Trust members and principal of Rabiammal ahamed Maideen womens college for Women, Tiruvarur -610001, Tamilnadu, India for providing necessary laboratory facilities to complete this manuscript.

## REFERENCES

1. Sukumar K., Perich M.J., Boober L.R., Botanical derivatives in mosquito control: a review, *Journal of the American Mosquito Control Association*, 1991; 7(1): 210–237.
2. Jacobson M. Review of neem research in the United States. In: Locke, J.C. and Lawson, R.H. (eds) proceedings of a workshop in neems potential in pest management program. USDA-ARS. Beltsville, MD. ARS 1990; 86: 4-14.
3. Verkerk, R.H.J. and Wright, D.J. Biological activity of neem seed kernel extract and synthetic azadirachtin against larvae of *Plutella xylostella*. *Pesticide science*, 1993; 37: 83-91.
4. Sary F, and Hans S. The National guides to medical herbs and plants. Tiger Books. Int. Plc. UK 1998.

5. Del Campo J, Amiot MJ, and Nguyen C. Antimicrobial effect of Rosemary extract. *J. Food Protect*, 2000; 63: 1359-1368.
6. Mann A Bansa A, and Clifford LC. An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*, Tanzania *J. Health Res*, 2008; 10(1): 34-38.
7. Marigold (*Calendula officinalis* L.). *Journal of Ethnopharmacology* 57: 139–144.
8. Mothana R.A and Lindequist U. Antimicrobial activity of some medicinal plants of the island Soqotra. *J. of Ethnopharmacology*, 2005; 96: 177-181.
9. Okigo, R. N.; Anuagasi, C.L. and Amadi, J. E. Advances in selected medicinal and aromatic plants indigenous to Africa. *Journal of Medicinal Plants Research*, 2009; 3(2): 86-95.
10. J. Clerk Maxwell, *A Treatise on Electricity and Magnetism*, 3<sup>rd</sup> ed., Oxford: Clarendon, 1892; 2: 68–73.
11. Kemper, K. J. (1999): *Calendula (Calendula officinalis)*. The Centre for Holistic Pediatric Education and Research cited in Safdar, W.; Majeed, H.; Naveed, I.; Kayani, W. K.; Ahmed, H Hussain, S. and Kamal, A.(2010): Pharmacognostical study of the Medicinal plant *Calendula officinalis* L.(Family Compositae). *International Journal of Cell & Molecular Biology*, 1(2): 108-11.
12. Talbert, R. *An Herb Society of America Guide*. The Herb Society of America cited in, 2015. [http://www.herbsociety.org/factsheets/Calendula Guide.pdf](http://www.herbsociety.org/factsheets/Calendula%20Guide.pdf)
13. Zitterl-Eglseer, K.; Sosa, S.; Jurenitsch, J., Schubert-Zsilavecz, M.; Loggia, D.R.; Tubaro, A.; Bertoldi, M, Franz C. Anti-oedematous activities of the main triterpene diol esters of marigold (*Calendula officinalis* L.). *Journal of Ethnopharmacology*, 1997; 57: 139–144.
14. Duke, J. A. *Handbook of Medicinal Herbs*. Boca Raton: Cancer Research center Press. 1991; 87-88.
15. Veera babu C., Radhika D., Mohaideen A., Indrani S and Priya R., Phytochemical and Biochemical profiles of *Calendula Officinalis* and *Azadirachta Indica* formulation cultured with organic manure, *International journal of current agricultural research*, 2015; 4(8): 131-133.
16. Roopashree, T. S.; Dang, R.; Shobha, R. H. and Narendra, C. Antibacterial Activity of Antipsoriatic Herbs: *Cassia Tora*, *Momordica Charantia* and *Calendula officinalis* Linn. *International Journal of applied research in natural products*. 2008; 1(3): 20-28.



17. Mullaicharam, A. R.; Amaresh, N. and Balasubramanian, H. Phytochemistry and Pleiotropic Pharmacological Properties of *Calendula officinalis* Linn.- A Review. *Journal of Pharmacognosy and phytochemistry*, 2014; 2(4): 1-10.
18. Farahpour, M. R. Antioxidant activity, anti nociceptive and anti-inflammatory effects of pot marigold hydroalcoholic extract on experimental animals. *International journal of pharmtech research*, 2014; 6(5): 1640-1646.  
<http://www.herbsociety.org/factsheets/Calendula Guide.pdf>
19. Muley, B. P.; Khadabadi, S. S. and Banarase, N. B. (2009) Phytochemical Constituents and Pharmacological activities of *Calendula officinalis* Linn.(Asteraceae). *Tropical Journal of Pharmaceutical research.*, 2009; 8(5): 455-465.
20. Kumar, A.; Singh, P. and Dora, J. Phytochemistry and Pharmacological activities of *Calendula officinalis*. *International Journal of Inventions in Pharmaceutical Sciences*, 2013; 1(1): 59-63.
21. Nand, P.; Drabu, S. and Gupta, R. K. Phytochemical and Antimicrobial Screening of Medicinal Plants for the treatment of acne. *Indian Journal of natural Products and Resources*, 2012; 3(1): 28-32.
22. Leffa, D. D.; Da Rosa, R.; Munhoz, B. P.; Mello, A. D.M; Mandelli, F. D.; Amaral, P. D.; Rossatto, A. E. and De Andrade, V. M. Genotoxic and antigenotoxic properties of *Calendula*. *Advances in li life sciences*, 2012; 2(2): 21-28.
23. Yiming L I, Seans L E, Zhang. The antimicrobial potential of 14 natural herbal plants. *J. Am. Dent. Assoc*, 2004; 135: 133-1141.
24. Senthilkumar P K, Reetha D, Screening of Antimicrobial Properties of Certain Indian Medicinal Plants. *J. Phytol*, 2009; 1: 193-1.
25. Periaswamy hemalatha, Devan elumalai, Arumugam Janaki, Muthu babu, Kuppan velu, Kanayairam velayutham and Patheri kunyil kaleena., Larvicidal activity of *Lantana camara aculeata* against three important mosquito species, *Journal of entomology and zoology studies*, 2015; 3(1): 174-181.