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DEVELOPMENT AND PHYSICOCHEMICAL STUDIES OF *MELIA AZEDERACH* EC AND ME FORMULATIONS

Shivam Dixit, Sandeep Kumar Dixit, Rajmani Prajapti, S. K. Raza, L. K. Thakur*

Institute of Pesticide Formulation Technology, Department of Chemicals & Petrochemicals, (Ministry of Chemicals and Fertilizers), Govt. of India, Sector-20 Udyog Vihar, Gurgaon (HR), India.

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For Correspondence:

L. K. Thakur

Shivam Dixit

Institute of Pesticide Formulation Technology, Department of Chemicals & Petrochemicals, (Ministry of Chemicals and Fertilizers), Govt. of India, Sector-20 Udyog Vihar, Gurgaon (HR), India.

E-mail:

thakurlk1964@rediffmail.com

shivamdixit@hotmail.com

ABSTRACT

Medicinal plants are widely used by human beings in control of microorganisms causing plant and human diseases due to their worldwide availability and fewer side effects. The plant *Melia Azedarach* L. (Bakain) belongs to the Meliaceae family and the different plant parts such as leaf, fruit, and young branches have been traditionally used by the medical practitioners for the treatment of malaria, diabetes, purgative, cough, skin disease, and so on in the Indian sub-continent. The Bio-efficacy studies prove that it has antioxidant, antimicrobial, anti-inflammatory, cardio-protective, analgesic, anticancer, antiulcer, antipyretic, antiplasmodial and male contraceptive properties. For the last few decades or so, extensive research work has been done to prove its biological activities and pharmacology of its extracts. The present study is focused on the preparation and Physicochemical studies of 2.5 & 5% (W/W) EC (Emulsifiable Concentrate) and ME (Microemulsion) prepared from the ethanolic extract of leaf part of *Melia Azedarach* Linn.

INTRODUCTION

Melia Azedarach L. belongs to the family Meliaceae. It is a deciduous tree that is native to northeastern India. It has several common names such as, White cedar, Persian lilac, Tulip cedar and Chinaberry. The plant has been introduced into several countries in Asia, North America and Latin America. In Jordan, it has been adopted as an ornamental plant. It has been known for quite a long time that the members of family Meliaceae are good sources of folk medications in Indian varieties [1]. In recent years, our scientific community has paid much more attention to finding an effective and environmentally friendly products to control pests and diseases [2-3]. Various parts of *M. azedarach* such as fruits, seeds, leaves of have shown many characteristics, biological activities against several harmful organisms [4]. The plant extract of *M. azedarach* showed efficacy against the *Rhipicephalus microplus,* the malaria vector *Anopheles stephensi,* the dengue vector, *Aedes aegypti* and the human lice *Pediculus humanus capitis* [5]. Many workers from across the world have been proven, the plant has potential insecticidal, acricidal, fungicidal and rodenticidal activity [6]. It has been also reported that the *M. azedarach* extracts can also exhibit the activity of NADPH-cytochrome c reductase and the cholinesterase on various insects [7]. Despite the all above uses the plant is also well known for its Antiviral, antibacterial and antifungal activity [8]. Thus, viewing the all above facts, we have decided to prepare various formulations of the ethanolic extract of this plant for the wider applicability. As a fact, the formulated products are much more pronounced for its use, handling and cost effective. In the present study, we have prepared EC (Emulsifiable Concentrate) and ME (Microemulsion) of different concentrations i. e. 2.5 & 5% (W/W). Various Physicochemical parameters of prepared formulations studied for its better applicability and use.

MATERIALS AND METHOD

Melia azederach plant material was collected from institute's premises (I.P.F.T., Gurgaon, HR, India). The leaves were first wiped to remove the adhering dust particles, air-dried and then ground into fine powder. All the chemicals *viz.* Dimethyl formamide, PEG-400, isopropyl-myrestate, C-IX, triton X-100, tween-80 and methyloleate were purchased from SD Fine chemicals pvt. Ltd., India.

Preparation of the plant extract:

Round bottom flask containing 380 ml of ethanol was fitted to the sauxhlet apparatus containing 120 grams of dried powder of *Melia azederach* and extraction was done in 72 hours. After the completion of the extraction, crude extract was filtered using Whatman no.1 filter paper and dried using a rotary evaporator to remove the ethanol.

GC-MS analysis of the plant extract:

The plant extract was analyzed for its essential components using GC-MS – QP 2010 plus (Shimadzu). Analysis was done by following parameters; (a) samplers- rinse with the solvent (pre run): 3.0, rinse with the solvent (post run): 5.0, plunger speed: high, syringe insertion speed high. (b) GC- column oven temperature: 50˚C, injection temperature: 250˚C, sampling time: 2 minute, pressure: 47.2 KPa, total flow: 13.1 mL/minute, column flow: 0.92 mL/minute and split ratio: 10.0, total run time 58.50 minutes. (c) MS- ion source: 250˚C, interface temperature: 280˚C and keeping solvent cut time: 6.5 minute.

The sample was found to be rich with various organic components and few of them are characterized based on m/z ratio obtained from its GC-MS analysis. Thus, the obtained m/z values were matched by NIST WILEY Mass Spectral Library for the final confirmation. Some of these characterized compounds are listed in table 1.

Table 1: GC-MS analysis of the ethanolic extract of *Melia Azedarach* L.

S. No.	Compound Name	Retention Time
1	1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTER	10.995
2	2(4H)-BENZOFURANONE, 5,6,7,7A-TETRAHYDRO-6-HYDROXY-4,4,7A-TRIMETHYL-, (6S-CIS)	13.162
3	ISOPROPYL TETRADECANOATE	13.728
4	2-PENTADECANONE, 6,10,14-TRIMETHYL-	14.053
5	HEXADECANOIC ACID, METHYL ESTER	15.337
6	1,2-BENZENEDICARBOXYLIC ACID, DIBUTYL ESTER	16.003
7	ETHYL 9-HEXADECENOATE	16.428
8	HEXADECANOIC ACID, ETHYL ESTER	16.545
9	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	18.645
10	METHYL 9-OCTADECENOATE	18.77
11	2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL	18.928
12	OCTADECANOIC ACID, METHYL ESTER	19.195
13	ETHYL (9Z,12Z)-9,12-OCTADECADIENOATE	19.928
14	9,12,15-OCTADECATRIEN-1-OL	20.07
15	OCTADECANOIC ACID, ETHYL ESTER	20.645
16	PHOSPHONIC ACID, DIOCTADECYL ESTER	27.42
17	NONADECANE	32.245
18	HEPTADECANOIC ACID, ETHYL ESTER	34.403
19	2,6,10,14,18,22-TETRACOSAHEXAENE,	35.045
20	EICOSANE, 7-HEXYL-	36.662
21	1-CHLOROHEPTACOSANE	40.878
22	VITAMIN E	41.437
23	STIGMASTEROL	43.762
24	STIGMAST-5-EN-3-OL, (3.BETA.,24S)-	45.012
25	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE	52.02

Formulation of EC:

For the preparation of 5% Emulsifiable Concentration (E.C.) Formulation, 0.50 gm crude plant extract was mixed with 0.50 gm DMF and 2.5 gm Triton X-100. The mixture was stirred well for 10 minutes. Finally, the volume of mixture was maintained up to 10gm using methyl-oleate (6.5 gm) [9].

Stability studies:

The stability studies of the 5% Emulsifiable Concentration (E.C.) Formulation were done using temperature ranges from 4˚C to 54˚C. No phase separation observed at extremes of temperature. It showed that the prepared EC is stable under varying temperature ranges.

TDS:

Total Dissolved Solid in the EC Formulation was analyzed using CyberScan PC 510 at 27˚C. The total TDS value was found to be 2.25 µS.

pH:

The pH of the EC formulation was measured by the same instrument at 20.7˚C. pH of the EC was found to be 5.70.

Formulation of microemulsion:

0.5gm of *Melia azederach* leaves extract with 1.5 grams DMF, 1.5grams of Triton X-100, Distilled water (6.5 grams) were mixed thoroughly to obtain a clear ME solution [10]. This microemulsion was used for further studies.

Stability studies:

The stability studies of the ME were done using temperature ranges from 4˚C to 54˚C. No phase separation was observed in either of the temperature. It shows that the prepared ME is stable under varying temperature ranges.

TDS:

Total Dissolved Solid in the microemultion formulation was analyzed using CyberScan PC 510 at 27˚C. The analysis of above prepared sample is a strong evidence of the formation of the 5% microemulsion of *Melia Azedarach* extract. The total TDS value was found to be 450µS.

Conductivity measurement:

In order to investigate the conductance, we have analyzed the conductivity of the prepared microemulsion formulation using CyberScan PC 510 at 27˚C. The sample was found enough to pass the conductance in its solution, which can be justified by its high conductivity value i.e. 31.4 mV.

pH:

The pH of the microemulsion formulation was measured by the same instrument at 27˚C. The pH of the microemulsion was found to be 6.14 which suggests its use as a topical drug bearing no risk to skin irritation in humans as the pH of the human skin lies in the range of 5.5-6.4.

Transmission Electron Microscope (TEM) Study:

In order to determine the shape and the size distribution of the ME, Transmission Electron Microscope (make-JEOL LTD) analysis was also carried out. A drop of the microemulsion solution was placed on a carbon-coated standard copper grid to obtain the TEM images.

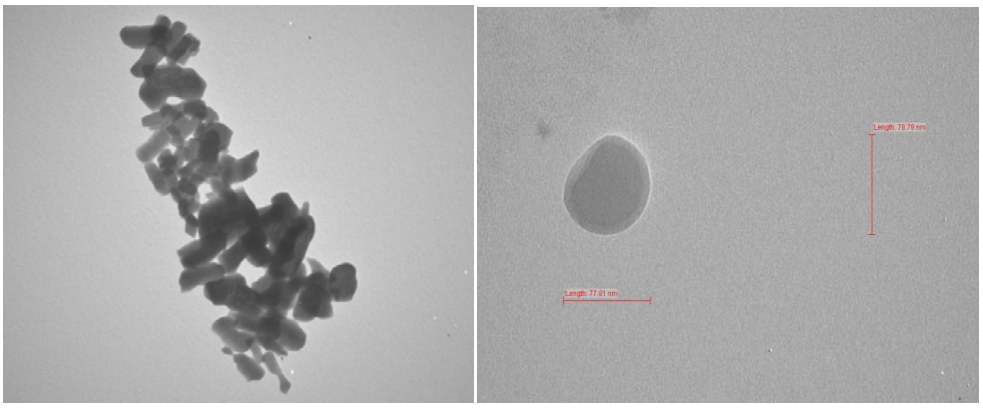

Figure 1: Transmission Electron Microscope (TEM) Image of the prepared ME.

RESULT AND DISCUSSIONS

A large number of chemical constituents are isolated and characterized from *Melia azederach*, which have promising biological activity [11]. There are a number of reports available related to the isolation, characterization and biological evaluation of the various extracts of the *Melia azederach* [12].In fact the ethanolic extract of this plant is found to posses a wide range of biological activity [13]. However, so far there is no systematic study available on the preparation of EC & ME formulation of *Melia azederach* plant extract. In the present study, we have prepared EC and ME of variable concentrations of ethanolic extract of *Melia azederach*. We have successfully prepared the 2.5-5.0% EC & ME and the 5% ME was characterized by TEM analysis. The particle size present in ME was found in the range of 77-80 nm. However the stability studies show that the prepared ME is stable at varying temperature ranges. The pH of the ME was found in the range of 6, which indicates that the prepared formulation can be used for various biological applications. However, the conductivity measurement of the ME is a good agreement for the various applications. The prepared EC was also studied for its pH and stability. All the above analysis of prepared EC shows that EC is stable at variable temperature range and pH is in the range of biological applications.

CONCLUSION

In the present study, we have successfully prepared EC & ME formulations from the ethanolic extract of the *Melia Azedarach* using different concentrations (2.5-5.0%). The prepared EC & ME formulations were analyzed for various Physicochemical parameters and all the values are in good agreement with standard values. Thus prepared formulations can be used for various biological applications. However, the biological studies of the formulations are under process.

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