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EXTRACTION ISOLATION CHARACTERIZATION AND PHARMACOLOGICAL SCREENING OF *AEGLE MARMELOS* FOR ANTIASTHMATIC ACTIVITY

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Keywords:

Aegle marmelos (L) *Correa*, Rutaceae, Anti-asthmatic activity

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ABSTRACT: *Aegle marmelos* (L) *Correa* also known as Bale, belongs to family Rutaceae. The plant is used to treat several diseases like bronchitis, asthma, malarial, astringent, laxatives, digestive and febrifuge. Leaves are used as antipyretic, antimicrobial, antidiabetic, antihistaminic, anti-inflammatory, antioxidant, and hyperlipidaemic activity. The plant contains diterpenoids, triterpenoids, and phenolic compounds. The present investigation was carried out to isolate, purify, and characterize active constituents from the *Aegle marmelos* (L) Correa plant. Powdered material of the whole *Aegle marmelos* (L) Correa plant was taken to prepare extract using Soxhlet apparatus. Various phytochemical tests were performed for alkaloids, glycosides, phenolic, flavonoids, *etc*. TLC was done for the isolation of compounds. GC-MS, thin layer chromatography, and Nuclear magnetic resonance further characterized the isolated compounds. The anti-asthmatic was evaluated by using *in-vitro* models. The Petroleum ether extract of *Aegle marmelos* (L) *Correa* significantly inhibited histamine contraction and confirmed the significant anti-asthmatic activity.

INTRODUCTION: Plants have been used as medicines for 1000 years. These medicines originally took the form of crude drugs such as powders, tinctures, teas, poultices, and other herbal formulations. Using plants as medicines has difficult to isolate energetic compounds, beginning with the isolation of morphine from the opium plant in the early 19th century. Isolation & Characterization of pharmacologically energetic compounds from medicinal plants continues today. Drug discovery from medicinal plants has evolved to various analysis methods and includes frequent fields of inquiry [1].

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Phytochemicals remain bioactive compounds initiated in plants that work through dietary fiber and nutrients to defend beside diseases. These phytochemicals are the secondary metabolites current in lesser quantities in difficult plants and they consist of Steroids, terpenoids, tannins, flavonoids, alkaloids and many others [2]. Herbal drugs are use of therapeutic herbs to avoid and treat diseases and disorders or to support health and curative. According to the World Health Organization (WHO), the usage of herbal medications worldwide exceeds that of the predictable drugs by two to three times [3].

Bronchial asthma is unique to the main respiratory disorders in clinical practice, produced by a combination of complex and incompletely silent environmental and genetic interactions. It is characterized by chronic airway infection and greater than before hyper-responsiveness leading to

chest tightness, wheeze, cough, and dyspnoea. However, India is an enormous country with immense economical, Socio-political, geographical, religious and racial diversity. In a survey of more than 2000 individuals, asthma was 2.0% in women and about 3.65% in men. The prevalence of current asthma was 11.9% in children. Boys had a significantly advanced occurrence of asthma compared to girls (12.8% and 10.7%, respectively) [4].

MATERIAL AND METHOD: The whole plant of *Aegle marmelos* (L) *correa* was collected in the month of September (2018) from near of, Peth-vadgaon, Tal-Hatkangale, Dist.-Kolhapur, Maharashtra, India and plant material was identified, confirmed and authenticated (authentication no. 289/2017-18 Dated-07 Nov 2017) by Prof. D. G. Jagtap, Head department of botany, Principal ShriVijaysinhaYadav Arts and Science College, Peth-Vadgaon.

After collection of plant material was thoroughly washed in distilled water and shade dried, crushed in an electrical grinder and then powdered.

Preparation of Plant Extract: The powdered material was subjected for extraction with various solvent in Soxhlet apparatus for 24 hrs based on increasing polarity in order of petroleum ether (60-800c) for defatting, chloroform, methanol and ethanol separately. All the extracts were concentrated and the solvent was evaporated in order to get dry extracts. The percentage yield of various extracts was calculated by the following formula-

% Yield = Weight of extract (g)/ Weight of dry powder (g) x 100

Phytochemical Screening: It was carried out by using standard chemical tests [5].

Isolation and Characterization of Individual Compounds: After chromatogram development, the plates were allowed to dry. Distance traveled by solute and solvent were marked and measured and their Rf value was calculated. Further from the data obtained from each extract's Rf values, the individual spots were isolated from the TLC plates. After drying the plates, each spot was scrapped from the plate using a sharp pointer. Each spot was collected in different test tubes according to their Rf values. They were further analyzed for their identity using analytical methods such as FTIR, NMR and GC-MS.

TABLE 1: SOLVENT SYSTEM FOR DIFFERENT EXTRACTS

Sr. no.	Extract	Solvent systems
1	Petroleum ether	Benzene: chloroform (10:10)
2	Ethanol	Chloroform: methanol (20:2)
3	Methanol	Benzene: chloroform (10:10)

Evaluation of Anti-asthmatic Activity [6]: The anti-asthmatic activity was evaluated using the isolated goat tracheal chain Preparation method, the goat tracheal tissue was obtained immediately after slaughter of animals. Pieces of trachea were collected in freshly prepared ice-cold oxygenated Krebs's solution, cut into individual rings, and tied together in series to form chain. One end was tied to the aerator and other attached isotonic frontal lever to kymograph paper on Sherrington rotating drum. Tissue was allowed to equilibrate for 45min. under to load of 400mg. A dose response curve of histamine was taken in variant molar concentration. After obtaining a dose curve of histamine on goat trachea aqueous solution of extract was added to reservoir and same dose histamine were repeated.

RESULT AND DISCUSSION:

TABLE 2: PERCENTAGE YIELD AND PHYSICAL APPEARANCE OF EXTRACTS

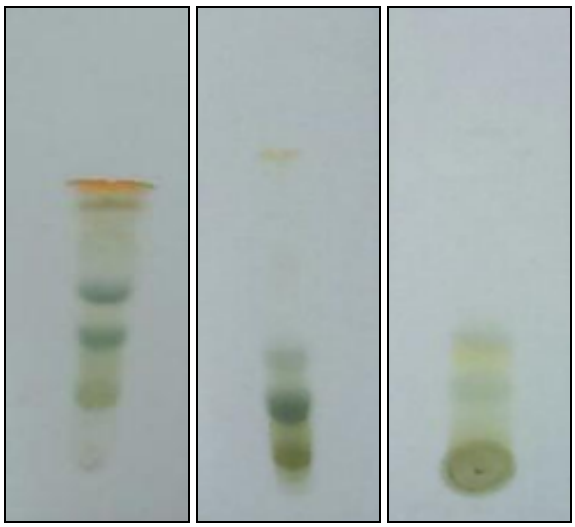
Sr. no.	Extract	% Yield (w/w)	Physical appearance
1	Petroleum ether	72.28	Dark greenish
2	Ethanol	78.68	Dark greenish
3	Methanol	87.2	Dark greenish

TABLE 3: PHYTOCHEMICAL TESTS OF *AEGLE MARMELOS (L) CORREA*

Test	Petroleum ether	Ethanol	Methanol
Test for carbohydrates			
Molishtest	+	+	+
Fehlingsolution Test	+	-	-
Benedict's test	-	-	+
Test for proteins			
Biuret test	+	+	-

Millon's test		-	+
Test for amino acid			
Ninhydrine test	+	+	+
Test for steroid			
Salkowaski test	+	+	+
Test for glycosides			
KellerKillani test	+	-	-
Foam test	+	-	+
Grignard reaction or sodium picrate test	+	-	-
Test for flavonoids			
Lead acetate test	+	+	+
Test for alkaloids			
Dragendroff's test	+	+	+
Mayer'stest	-	+	+
Hager test	-	-	-
Wagner's Test	-	+	-
. Test for tannins and phenolic compounds	+	-	+

(-) indicates absence, (+) indicates present.



(1) (2) (3)

FIG. 1, 2, 3: TLC PROFILE OF PETROLEUM ETHER EXTRACT (1), ETHANOL EXTRACT (2), METHANOL EXTRACT (3) RESPECTIVELY

TABLE 4: TLC PROFILE OF DIFFERENT EXTRACTS OF *AEGLE MARMELOS* (L) *CORREA*

Extract	Spot	R_f value
Petroleum ether	A	0.77
	B	0.74
	C	0.68
	D	0.51
	E	0.20
Ethanol extract	A	0.90
	B	0.53
	C	0.41
	D	0.31
Methanol extract	A	0.56
	B	0.49
	C	0.40

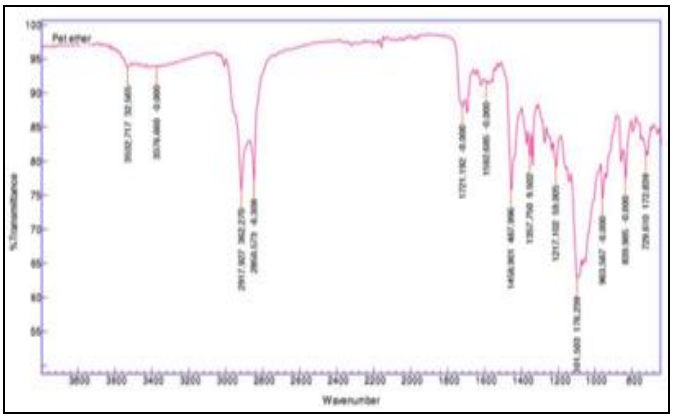


FIG. 4: FTIR SPECTRA OF ISOLATED COMPOUND (A) OF *AEGLE MARMELOS (L) CORREA*

Characterization of Isolated Compounds by Spectral Analysis:

Isolated Compound A:

FTIR Spectra:

Interpretation of FTIR spectra confirms the presence of C=O Aromatic ketone, O-H, C-H stretching, C=C Stretching.

Mass Spectra:

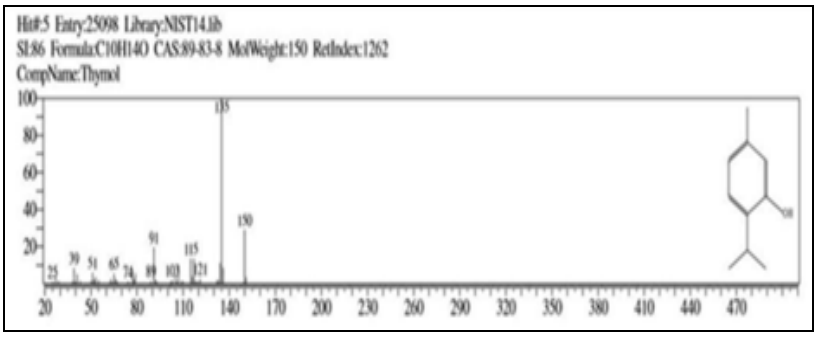


FIG. 5: MASS SPECTRA OF ISOLATED COMPOUND (A) OF *AEGLE MARMELOS* (L) *CORREA*

Interpretation of Mass spectra confirms the presence of Thymol

NMR Spectra:

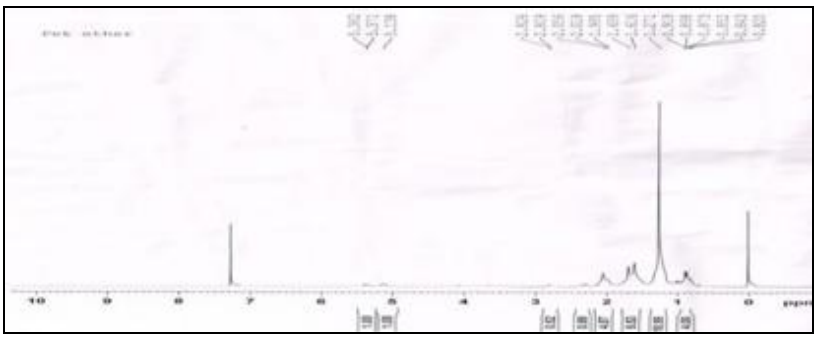


FIG. 6: H^1 NMR SPECTRA OF ISOLATED COMPOUND (A) OF *AEGLE MARMELOS (L) CORREA*

Interpretation of NMR spectra shows the result close to the Thymol from the above characterization study isolated compound A was identified is Thymol.

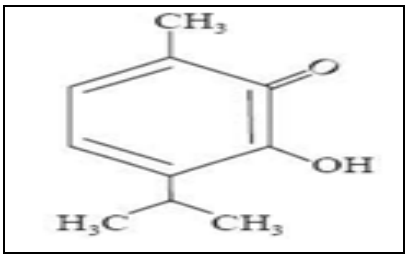


FIG. 7: THYMOL

Isolated Compound B:
FTIR Spectra:

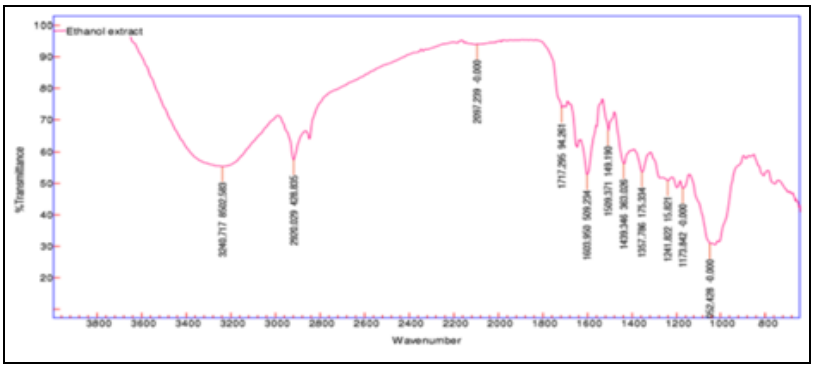


FIG. 8: FTIR SPECTRA OF ISOLATED COMPOUND (B) OF *AEGLE MARMELOS (L) CORREA*

Interpretation of FTIR spectra confirms the presence of O-H, C-H stretching, C=C Stretching

Mass Spectra:

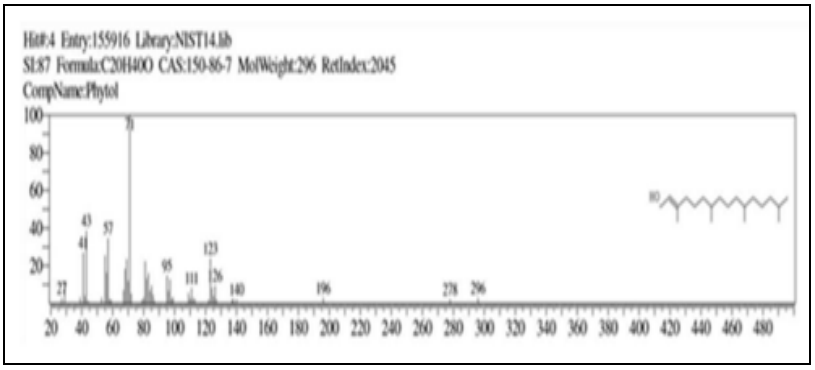


FIG. 9: MASS SPECTRA OF ISOLATED COMPOUND (B) OF *AEGLE MARMELOS (L) CORREA*

Interpretation of Mass spectra confirms the presence of Phytol.

NMR Spectra:

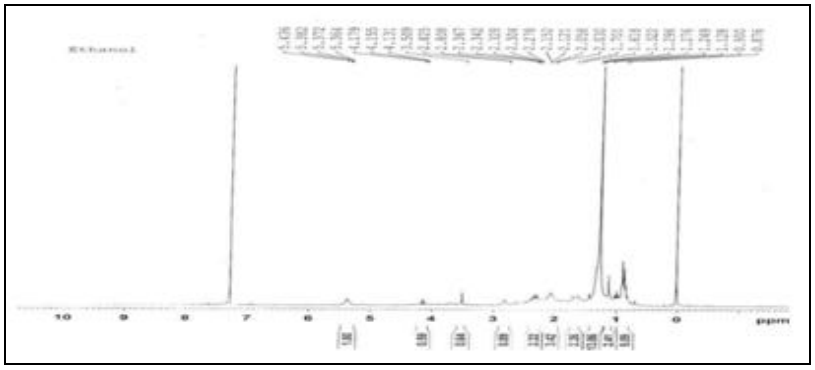


FIG. 10: H^1 NMR SPECTRA OF ISOLATED COMPOUND (B) OF *AEGLE MARMELOS (L) CORREA*

Interpretation of NMR spectra shows the result close to the Phytol from the above characterization study isolated compound B was identified is Phytol.

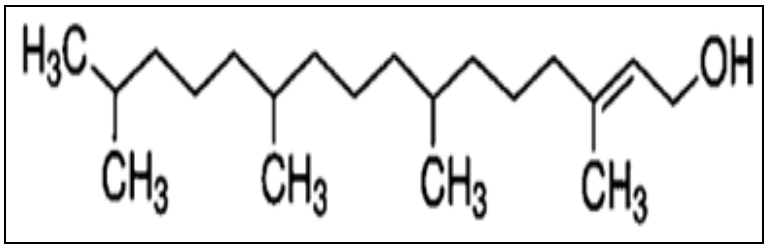


FIG. 11: PHYTOL

Pharmacological Screening by *In-vitro* Evaluation of Anti-asthmatic Activity: Responses of 2.5 µg/ml histamine was found to be Mean ±SD = 15.540±0.45607.

TABLE 5: EFFECTS OF DIFFERENT EXTRACTS OF *AEGLE MARMELOS (L) CORREA*ON HISTAMINE INDUCED CONTRACTION ON ISOLATED GOAT TRACHEAL CHAIN PREPARATION

Sr. no.	Drug	Dose	Height (Mean±SEM)
1	Histamine	2.5	15.540±0.45607
2	Petroleum ether extract	300	10.680±0.6300
3	Ethanol extract	300	4.700±0.4062
4	Petroleum ether extract	400	7.960±0.7231
5	Ethanol extract	400	3.600±0.5431

n= 5, Values are in Mean± SEM, Data was analysed using one-way ANOVA.

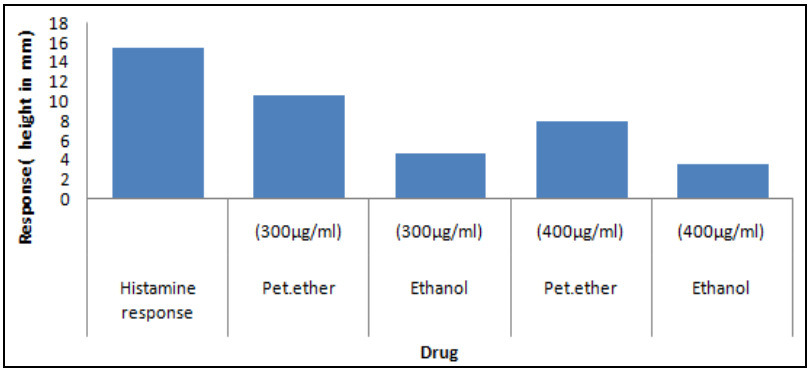


FIG. 12: EFFECTS OF DIFFERENT EXTRACTS OF *AEGLE MARMELOS (L) CORREA* ON HISTAMINE INDUCED CONTRACTION ON ISOLATED GOAT TRACHEAL CHAIN PREPARATION

DISCUSSION: In the present study Petroleum ether extract (300 µg, 400 µg) of *Aegle marmelos* (L) *correa* showed significant dose-dependent antiasthmatic activity in goat tracheal chain model. While Ethanolic extract (300µg, 400µg) showed satisfactory activity.

CONCLUSION: The plant *Aegle marmelos* (L) *correa* seems to be a promising candidate with respect to its anti-asthmatic activity and may be recycled as adjuvant to dietary therapy and drug treatment for controlling asthma. We evaluated in-vitro anti-asthmatic activity using the goat tracheal chain model we concluded that the Aegle marmelos (L) Correa is a potential member for the anti-asthmatic activity.

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CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

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