Maytenus emarginata: A Desert plant

This book Maytenus emarginata: A Desert Plant gives a detail study on the importance of this plant in sense of various scientific, economic, social and ethical aspects. There is a systematic analysis of the in vivo and in vitro comparative studies of tissue culture, primary and secondary metabolites, antimicrobial and antioxidant activities by using different plant parts like: fruit, leaf, root, stem and callus also.
In this book the fundamental concepts of tissue culture and biochemical compounds have been introduced and analyzed in a simple manner. The contents of the book will be of interest to all research scholars, industries, pharmacy etc.
Key features of the book include a simple approach with illustrations, tables , pictures and graphs. The basic terminologies have been defined in the text while introducing the topics and some useful terms mentioned in the text have been explained in the glossary for an easy grasp by students and research scholars.

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Maytenus emarginata: A Desert plant

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Dedication

This work dedicated to my lovely daughters Aagmya Maan (kishmish) & Aaradhya Maan (Veer) who deserves real credit as she suffered a lot due to the absence of my company throughout the tenure of this work. He is my inspiration in everything I do & every choice I make.

CONTENTS

INTRODUCTION

In India, there are many traditional system of medicine are developed in which **Ayurveda** and **Sidha** are well known and now commonly called **Indian System of Medicine**. Treatments based upon medicinal herbs and herbo mineral combinations are essence of Ayurveda and Sidha. Modecinal plants coming under ayurveda listed as Ayurvedic Medicinal Plants. According to **WHO** (World Health Organization), due to low expense 80 % of Asian and African population uses herbal medicine, near about 7,000 medical compounds and 120 active compounds currently isolated from herbs and approximate 25% of drugs using in USA derived from medicinal plants.

India's traditional medical system are part of a time honored and time tested culture that still intrigues people today. They successfully recommend the use of nature to treat primary and complex ailments for over 3,000 years and obviously have a contemporary relevance. In an age when toxic drugs are increasingly unwelcome and when thinking people are using viable alternatives, India's medical heritage is incredible. There has been resurgence in the consumption and demand for plants. Plants are finding use as pharmaceuticals, neutraceuticals, cosmetics and food supplements.

Considering all these beneficial aspects and medicinal value of plants, present investigation involves one economically and medicinally important plant *Maytenus emarginata*. This plant was identified from the Herbarium of Botany Department, university of Rajasthan, Jaipur. The RUBL number of *Maytenus emarginata* is 20908. According to verification and identification the following details are available as follows:-

The genus *Maytenus* was established in 1782 by Molina. It consists of about 300 species distributed mainly in tropical and subtropical areas, with some found in warm temperate zones.

Table 1.1 Traditional medicinal uses of *Maytenus* species.

S.no	Maytenus Species	Medicinal uses
1.	*M. acuminata (Lf.)*	Stomach ailments
2.	*M. arbutifolia*	Malaria
3.	*M. aquifolium Mart.*	Ulcers
4.	*M. buchananii*	Stomach upsets
5.	*M. heterophylla*	Diarrhoea, Coughs, Colds, Haemorrhoids, Measles, Venereal diseases, Antihelmintic, Hernia, Syphilis, Tonic, Dysmenorrhoea
6.	*M. ilicifolia Mart.*	Ulcers
7.	*M. putterlickiodes*	Hookworm
8.	*M. senegalensis*	Chest pains, Rheumatism, Snake bite, Eye infections, Aphrodisiac, Pneumonia, Tuberculosis, Venereal diseases, Epilepsy, Pain, Constipation, Infertility, Sore throat, Earache, Tumours, Dysentery, Snakebites,
9.	*M. undata*	Malaria, Tonic, Syphilis, Urethra infections

***Maytenus emarginata*: The Desert Plant**

BOTANICAL NAME: *Maytenus emarginata* (Willd.) Ding Hou

SYNONYMS

Celastrus emarginatus Willd.

Gymnosporia emarginata (Willd.) Thw.

Gymnosporia Montana (Roth) Benth.

BOTANICAL CLASSIFICATION

Kingdom	:	Plantae
Phylum	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Celastrales
Family	:	*Celastraceae*
Genus	:	*Maytenus*

COMMON NAMES

Hindi	:	Vinger, Kankero, Baikal, Malkangni
English	:	Thorny staff tree
Sanskrit	:	Vikankata, Sudhavridsha
Bengali	:	Bakundri, Vaichigacha
Kannada	:	Tandrasi, Tandraja, Haalu manike, Malega, Malkaamguni
Malayalam	:	Katou-patsjotti, Kanguni
Marathi	:	Baefal, Baekar, Bhaaroolee, Bharatti, Bharuli, Harmocha, Vekal, Vekar, Yekkadi
Tamil	:	Kattangi, Nandunarani, Valulu-Vai
Telugu	:	Chinni, Danthi, Sinni, Danti, Chinni tuppa, Chinni chettu, Goddali-cippa

Gujarati : Vickado

DISTRIBUTION

The species is globally distributed in Paleotropics. Within India, it is common in dry scrub forests throughout, particularly on poor soils in Central and Western peninsular India. The genus *Maytenus* distributed in drier parts of Central, South-Western and North-Western India. It is found throught in India (Madhya Pradesh, Uttar Pradesh, Punjab, Maharashtra, Gujarat, Delhi, Bihar, Tamilnadu, and Rajasthan.

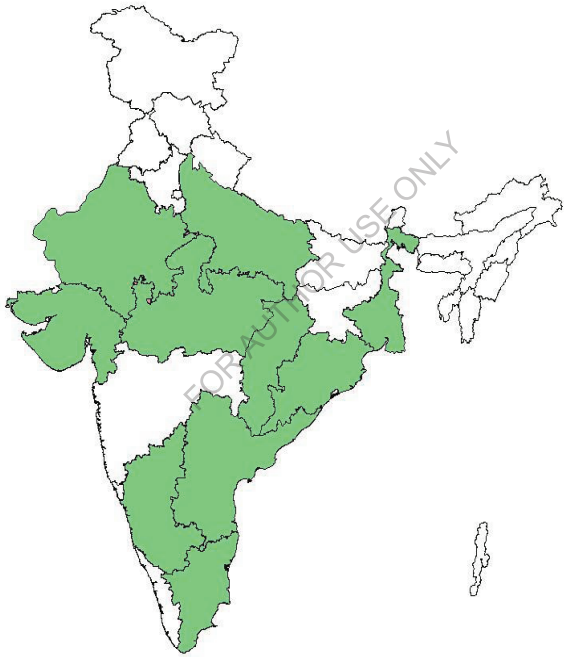


Figure-1: Distribution map of *Maytenus emarginata* plant in India.

In Rajasthan its found in Ajmer: Ajmer-Udaipur road, kota: Shahabad, Pali: Gurupratap singh village, Sirohi: Vadakhoda, Tonk: Rajmahal, Doogor Beed (Nagaur), Fahelpur Beed (Sikar), Siker Beed (Siker), Nokha (Bikaner), Karni Mata

Oran, Deshnoke (Bikaner), Shri Balaji (Nagaur), Chohta (Barmer), Shri Mukam-Nokha (Bikaner), Khejarali (Jodhpur), Nursery of Rajasthan University, World Forestry Arboretum,Jhalana Dungri (Jaipur).

ECOLOGY

Growing at elevations from near sea level and locally abundant on the coast on sand at the edge of mangrove forest or secondary forest, at forest margins, in thickets on branches and hillsides and on sea cliffs, often on limestone. Long, hot summers are needed for production of flowers and fruits.

BOTANICAL DISCRIPTION

The members of Celastraceae family generally are trees and shrubs comprising about 55 genera and 850 species that are sometimes climbing or vining (Gonzalez *et al*., 2000). *Maytenus emarginata* is a small, compact tree, 3-5 meter high; young branches purple, often spiny, with leaves and flowers on the spines. All the botanical descriptive characters of this plant are listed in Table 1.2 and Figure- 2.

Figure- 2: line diagram of different plant parts of *Maytenus emarginata* (Willd.)

(A) Flowering twig (B) and (C) Flower (D) Stamens (E) L.S of flower (F) T.S of ovary

Table-1.2: Botanical Description of *Maytenus emarginata* (Willd.)

Plant type		Evergreen, Dioecious tree, Very hardy plant.(Fig. 3 A) Habit:- Scandent shrub or small tree Name code:- 202785
Growing requirements		Light requirement: - Full sun with midday shade Soil tolerances: - Sandy soil, limestone Best season:- Monsoon Drought tolerance:- Various types of stresses of the desert
(a)	**Leaf**	Thick, coriaceous and usually longer than 40mm, apex rounded, alternate on young branchlets, fasciculate on older ones. (Fig. 3B)
(b)	**Flower**	Bisexual or sometimes functionally unisexual, actinomorphic, white or cream colored 5-7mm in diameter. Male flower:- Stamens slightly shorter than petals, stigmas absent, disc green, 5-lobed Female flower: - Staminodes shorter than stamens of male flower, ovary 3-locular, green, style as long as ovary. (Fig.3 C)
(c)	**Fruits**	Capsule, berry, 6-12mm long, green becoming red. (Fig. 3D)
(d)	**Seeds**	Seeds are 1-4, with basal aril or the aril partially to completely covering the individual (Fig. 3E)
(e)	**Root**	Tap root, Cream yellow in color. (Fig.3 F)
(f)	**Bark**	Pale brown, smooth, cracked. (Fig. 3G)
Pest and diseases		Trouble free

Figure-3: Different plant parts of *Maytenus emarginata* (Willd.) Ding. Hou

(A) Healthy *Maytenus emarginata* plant. (B) leaf (C) Flowers (D) Fruits (E) Seeds (F) Root (G) Bark

GROWTH PATTERNS

Maytenus emarginata (Willd.) grow in moderately fertile, moist but well-drained soil in full sun with midday shade. Fruit appear in November to February and flowers appears in October to June. Develops new leaves from June to august and fruit ripens from March to April as shown in Table-1.3. The best season for collecting of this plant for medicinal uses is monsoon.

Table -1.3: General growth pattern of *Maytenus emarginata* (Willd.)

Plant Parts	Months											
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
New leaf												
Fruit												
Flower												
Fruit ripen												

CHEMICAL CONSTITUENTS

Over the last 30 years or so, a large number of secondary metabolites exhibiting a wide range of bioactivity have been extracted from the Celastraceae. The bioactive metabolites of the genus Maytenus in plants belongs to family Celastraceae, which are widely used as folk medicines in South America (Takimoto and Calvo, 2008).

Plant Parts	Compounds

Root	Quratea proanthocyanidin A, Emarginatine A,
Stem	B-sitosterol, β-sitosterol- glycoside, Emarginatine A, Emarginatinine, Tingenin A, Tingenin B, Emarginatine D, Emarginatine E, Emarginatine F, Emarginatine G.
Leaf	β-sitosterol, Kaempferol, Luteolin, Trifolioside, Kaempferol tetraglycoside, Kampferol diglycoside, Kampferol triglycoside, Quercetin triglycoside, Quercetin tetraglycoside
Fruit	Kaempferol, Luteolin, Quercetin

MEDICINAL VALUE

Traditionally species of *Maytenus* has been used for fever, asthama, rheumatism and gastrointestinal disorders worldwide. Recently some biomolecules from *Maytenus* species has been reported to be active against HIV-Protease (Hussein *et al*., 1999) Carcinoma and leukemia (Tin-wa *et al*., 1971) Ulcers (Vilegas *et al*., 1999) and MDR (Multi Drug Resistance) (Spivey *et al*., 2002). Various parts of this plant contain immense medicinal properties which are mentioned under:

Plant Parts	Medicinal value
Root	Used in gastrointestinal troubles, especially dysentery (Kothari and Londhe ,2000).
Stem	Tender shoots of the plant help for mouth ulcer. The bark is ground to a paste and applied with mustard oil to kill lice in the hair.
Leaf	Pulverized leaves of *Maytenus emarginata* are given in milk to children as a vermifuge (Pullaiah ,2006). A decoction of the leafy twigs is used as a mouthwash to relieve toothache. Ash of leaves used to heal up sores and wound gives cooling effect. The leaves are burnt and mixed with ghee to form an ointment used to heal sores (Agrawal and Nag. 2009). The tender leaves are chewed raw in the treatment of jaundice.

Fruit	The fruits are used in medicines to purify blood (Bhandari, 1990).

BIOLOGICAL ACTIVITIES REPORTED FOR GENUS *MAYTENUS*

(i) Antiparasitic activity

Hot water extract of leaf and/or root bark *of M.cuminata, M. heterophylla* and *M. senegalensis* showed moderate (33%-49%) activity against chloroquine-resistant *Plasmodium berghei* NK65 *in vivo*. Leaf extract of *M. heterophylla* and root bark extract of *M. senegalensis* showed synergistic effect with chloroquine and improved parasitaemia suppression in the range of 38%- 66% (Muregi *et al*., 2007). Methanol extracts of *M. acuminata* leaf and root barks possessed statistically significant parasitaemia suppressions of 31.7- 59.3%. In combination with chloroquine methanolic extracts of *M.senegalensis* leaf and root bark gave statistically significant and improved suppressions ranged from 45.5 to 85.1%(Muregi *et al*., 2006).

(ii) Antibacterial and antifungal activities

Ethanol extract of root bark of *M. macrocarpa* possessed broad spectrum antibacterial activity against *Bacillus cereus* (MIC= 125µg\ml), *Bacillus subtilis* (MIC= 125µg\ml), *Bacteroides fragilis* (MIC= 250µg\ml), *Enterococcus faecalis* (MIC= 250µg\ml), *Escherichia coli* (MIC=125µg\ml), *Pseudomonas aeruginosa* (MIC= 250µg\ml), *Staphyllococcus aureus* (MIC= 250µg\ml), *Staphyllococcus epidermidis* (MIC= 125µg\ml), *Streptococcus pyogenes* (MIC= 125µg\ml) and *Candida albicans* (MIC= 250µg\ml) (Kloucek *et al*., 2006). This was confirmed by the isolation of different compounds showing antibacterial activities from this plant. Scutione was isolated from this plant and possessed antibacterial activity against gram positive bacteria. Aqueous, hexane and methanol extracts of *M. senegalensis* showed potent antibacterial activity against Gram-positive bacteria as well as some anti-inflammatory activity (Matu and Staden, 2003).

(iii) Antioxidant activity

Ethanolic extract of *M. ilicifolia* root bark possessed a potential antioxidant activity against the monocation 2,2'- azinobis(3-ethylbenzothiazoline-6-sulfonic acid) and HOCl radical (Bruni *et al*., 2006; Vellosa *et al*., 2006). The flavanonols 4'-*O*-methyl-(-)-*epi*gallocatechin and (-)- *epi*catechin , the flavonol glycosides; quercetin glycosides; and the proanthocyanidine; Oureata proanthocyanidin A showed significant activity (Corsino *et al*., 2003).

(iv) Gastro-intestinal protection actions

A tea (infusion) of *M. ilicifolia* containing a polysaccharide consisting of arabinose, galactose, galacturonic acid, 4-O-methylglucuronic acid, rhamnose, and glucose in a 42:41:6:5:4:2 molar ratio as a major component inhibited ethanol-induced gastric lesions in rats with an ED50 of 9.3 mg/kg (Cipriani *et al*., 2006). The dried extract of *M. ilicifolia* in doses of 140, 280 and 420 mg/kg i.p. produced a significant reduction in the ulceration index, as well as a significant increase in the volume and in the pH of the gastric secretion in male wistar rats (Tabach *et al*., 2003). Compared with cimetidine, the lyophilized aqueous extract of *M. ilicifolia* leaves (7-28 mg %) reduced acid secretion in the isolated frog gastric mucosa by antagonizing histamine H2-receptors (Ferreira *et al*., 2004). Spray dried extract of *M. aquifolium* was found to elicit significant antiulcer activity against acidified-ethanol, indomethacin and acute stress-induced ulcers (Bersani-Amado *et al*., 2000). Many compounds were tested as potential antiulcers. Triterpenes isolated from many *Maytenus* species produced significant antiulcer activity. Friedelin and 3β- Friedelanol possessed antiulcer activities (Mossi *et al*., 2004).

(v) Effects on the CNS

The chloroform extract of *M. obtusifolia* caused a decrease in spontaneous activity and induced catalepsy in mice up to 240 min. The extract significantly protected the mice against amphetamine-induced toxicity and decreased the conditioned response in rats, in a dose-related manner (Neuroleptic-like effects)

(De Sousa and De Almeida, 2005).

(vi) Anti-inflammatory and analgesic activities

Variety of compounds isolated from this genus also showed anti-inflammatory activity supporting the above mentioned activities of these plants. The friedelin triterpenes; polpunic acid (Buffa Filho *et al*., 1981) and 20-Epikoetjapic acid (Martin, 1973) possessed anti- inflammatory activity. Quinone methide triterpenes; tingenone (Dymowski and Furmanowa, 1990) 22β-hydroxy-tingenone , the flavononols; 4'- *O*-methyl-(-)-*epi*gallocatechin (Corsino *et al*., 1998) and ouratea proanthocyanidin A (Gonzalez et al., 1982) were found to have anti-inflammatory activity.

(vii) Cytotoxic and antitumor activity

The triterpene; canophyllol and the dihydro-β-agarofuran pyridine alkaloids mayteine (Nakagawa *et al*., 2004) produce their antitumor effect through induction of cytokines whereas eumaitenin was found to produce its antitumor activity through chromosome aberration (Garcia *et al*., 1993). On the other hand, the triterpene; maytenfoliol (Nozaki *et al*., 1986) and maytenfolic acid (Nozaki *et al*., 1982) and the ansa macrolides; maytanbutine (Kupchan, 1977) and normaytansine (Sneden and Beemsterboer, 1980) were also found to possess antileukemic activity. Several compounds isolated from different *Maytenus* species produced cytotoxic and antitumor effects like: the triterpenes glochidonol (Nunez *et al*., 2005), nepeticin (Gonzalez, 1982), celastrol, macrocarpin A (Chavez *et al*., 2000), macrocarpin A acetyl derivative maytenin, 20α-hydroxy-6-oxoiguesterol, 22β-hydroxy-6- oxoiguesterol ((Buffa Filho *et al*., 1981; Shirota *et al*., 1994), pristimerin, the dihydro-β-agarofurans; the dihydro-β-agarofurans pyridine alkaloids; emarginatine A (Sugira *et al*., 1973), emarginatine E , emarginatine B and emarginatine F (Kuo *et al*., 1994).

(viii) Contraceptive effects

The lyophilized hydroalcoholic extract of the leaves of *Maytenus ilicifolia* caused a pre-implantation embryonic loss; an abortifacient effect which may be

due to interfering with the uterine receptivity to the embryo. Neither morphological alterations of the reproductive system nor an embryotoxic effect were found (Montanari and Bevilacqua, 2002).

(ix) Insecticidal and insect antifeedant activities

Dihydro-β-agarofuran sesquiterpenes are the most important group of compounds as insecticides. They were found to produce antifeedant and growth inhibitory activities. Dihydro-β-agarofuran sesquiterpene polyesters eumaitenin (Gonzalez *et al*., 1982), isoalatol (Gonzalez *et al*., 1996) and its derivatives were assayed for their antifeedant activity on larvae of the Egyptian cotton leaf worm *Spodoptera littoralis* (Bisduval), using the leaf disk method. The dihydro-β-agarofuran sesquiterpene pyridine alkaloids; wilfordine , alatusinine and euonine exhibited strong antifeedant activity against *Spodoptera littoralis.*

(x) Antidiabetic activity

Lambertic acid is a diterpene isolated frombark of *M. laevis*. It has antidiabetic effect through inhibition of aldose reductase (Matsuda *et al*., 1999).

Therefore, in view of the significant medicinal potential of the *Maytenus emarginata* (Willd.) was selected in the investigation for the systematic evaluations. viz. –

- To assess an attempt to develop an efficient protocol for callus induction from the *M. emarginata* in order to derive more economic and conservative ways to obtain phytochemicals.
- To assess the biosynthetic potentialities undertaking the quantification of various primary and secondary metabolites.
- To assess the antimicrobial and antioxidant activity, so as to certain the pharmacological potential of the selected plants.

Accordingly, the work has been classified as follows-

(I) CALLUS INDUCTION

In the present investigation, unorganized callus cultures of *M. emarginata* were established on MS medium supplemented with plant growth hormones. In *M. emarginata*, it was the combination of 2, 4-D (3.0 mg/L) and Kn (0.1 mg/L) which induces dark green compact callus but in also, the stock callus was maintained at the addition of additives *viz;* (50 mg/L) ascorbic acid, (25 mg/L) citric acid, (25 mg/L) adenine sulphate and (25 mg/L) arginine. For biochemical investigation, the unorganized callus tissue was harvested at maximum GI (Growth index) and analyzed for primary and as well as secondary metabolites estimation.

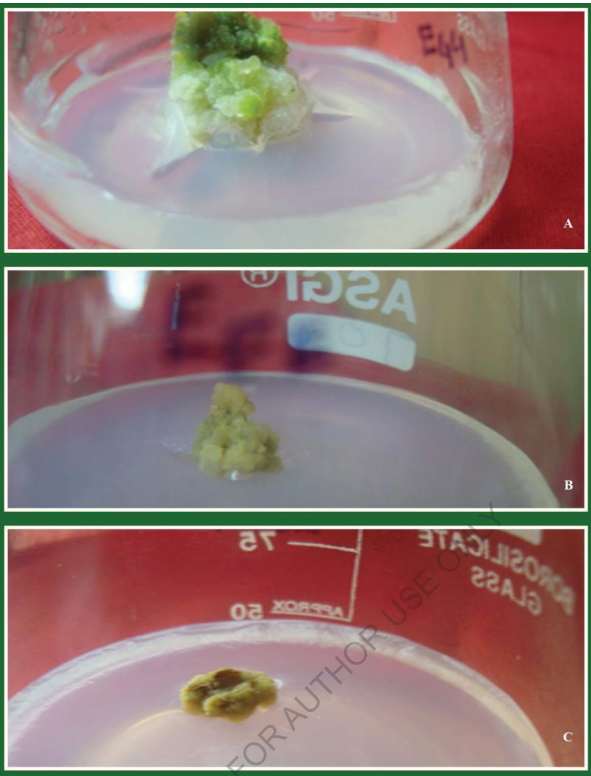


Fig. A-C : Callus induction from leaf explant of *Maytenus emarginata* (Willd.) on MS medium supplemented with various auxins.

Fig. A : Effect of 2, 4-D (0.1-8.0mg/L)
Fig. B : Effect of NAA (0.1-8.0mg/L)
Fig. C : Effect of IAA (0.1-8.0mg/L)

Fig. A-B : Effect of various cytokinins in combination with 2, 4-D on callus development in *Maytenus emarginata* (Willd.).

Fig. A : Effect of BAP (0.05 - 2.0 mg/L)
Fig. B : Effect of Kn (0.05 - 2.0 mg/L)

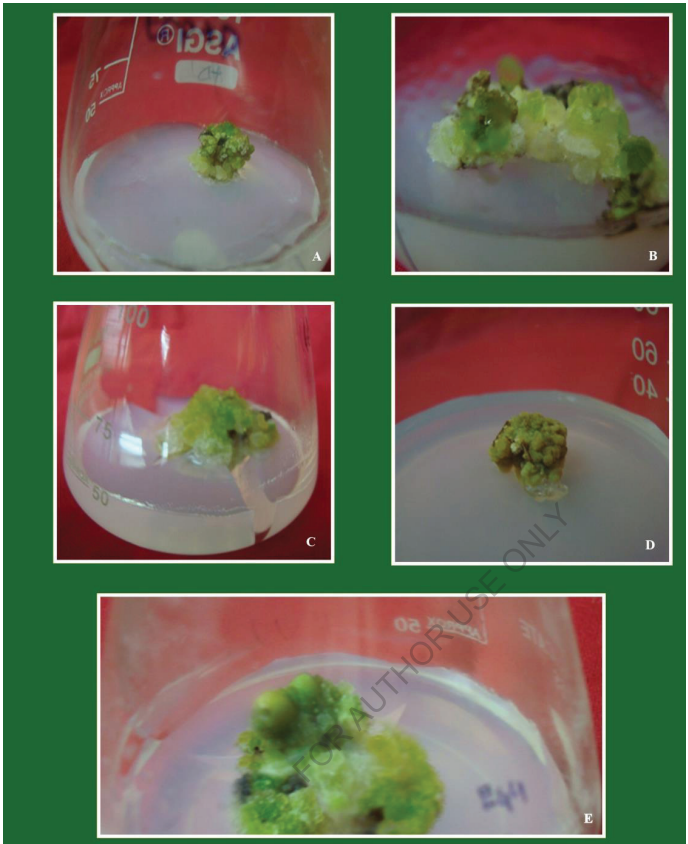


Fig. A-D : Effect of additives in combination with 2, 4-D, Kn on callus development in *Maytenus emarginata* (Willd.).

Fig. A : Effect of ascorbic acid (15-100 mg/L)
Fig. B : Effect of citric acid (15-100 mg/L)
Fig. C : Effect of adenine sulphate (15-100 mg/L)
Fig. D : Effect of arginine (15-100 mg/L)
Fig. E : Effect of ascorbic + citric +adenine +arginine (15-100 mg/L)

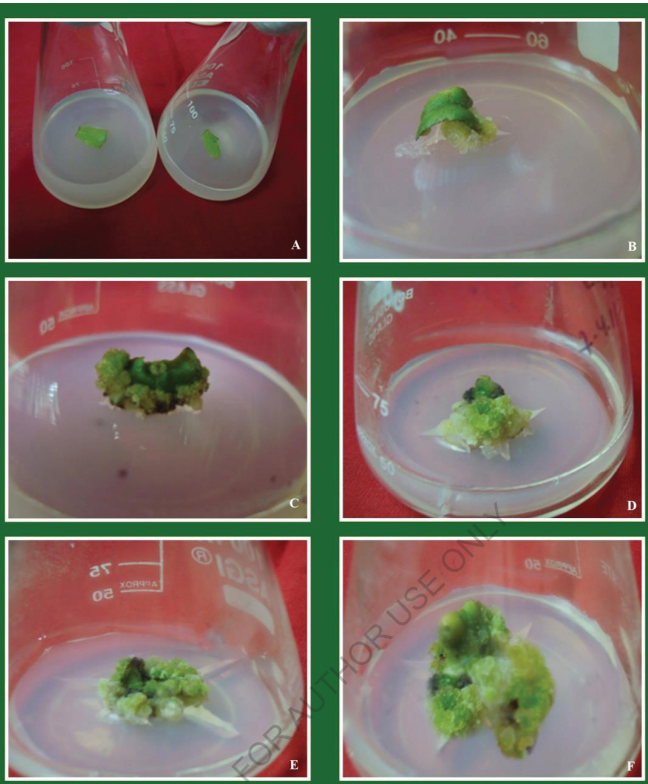


Fig. A-F : Various stages of callus induction of *Maytenus emarginata* (Willd.) Ding. Hou.

Fig. A - Inoculate leaf explant on MS medium supplemented with (2, 4-D+Kn+citric acid+ascorbic acid+ adenine sulphate+arginine)
Fig. B - Callus initiation
Fig. C - After 2- Week
Fig. D - After 4- Week
Fig. E - After 6- Week
Fig. F - After 8- Week

(II) BIOCHEMICAL ANALYSIS

(i) Primary metabolites

In the present study, various plant parts (root, stem, leaves, fruits and callus) *M. emarginata* were investigated for primary metabolites even if all the metabolite were present in appropriate and in trace concentration in all plant parts *in vivo* and *in vitro*. During present research work, estimation of different kind of metabolites like carbohydrate, protein, lipid, total phenol, amino acid and ascorbic acid have been estimated in this plant.

Carbohydrate

In *M. emarginata* Quantitative estimation of sugar shows that content of sugar is more in fruit i.e 45.80 mg/gdw and minimum in stem i.e. 18.40 mg/gdw. The maximum amount of starch was found in root (82.80 mg/gdw) and minimum amount was observed in fruit (26.17 mg/gdw).

Protein

In *M. emarginata* maximum amount of protein was observed in fruit (30.88 mg/gdw) and minimum amount was observed in stem (14.20 mg/gdw).

Lipids

The maximum amount of lipids was found in fruit (95.40 mg/gdw) and minimum in callus (40.24 mg/gdw) of *M. emarginata.*

Phenol

In *M. emarginata* total levels of phenols were found to be higher in root (9.26 mg/gdw) and minimum in stem (5.16 mg/gdw).

Amino acid

In *M. emarginata* the maximum amount of amino acid was found in leaf

(13.12 mg/gdw) and minimum in callus (6.73 mg/gdw).

Ascorbic acid

In *M. emarginata* the maximum amount of ascorbic acid was found in leaf (13.38 mg/gdw) and minimum in callus (4.53 mg/gdw).

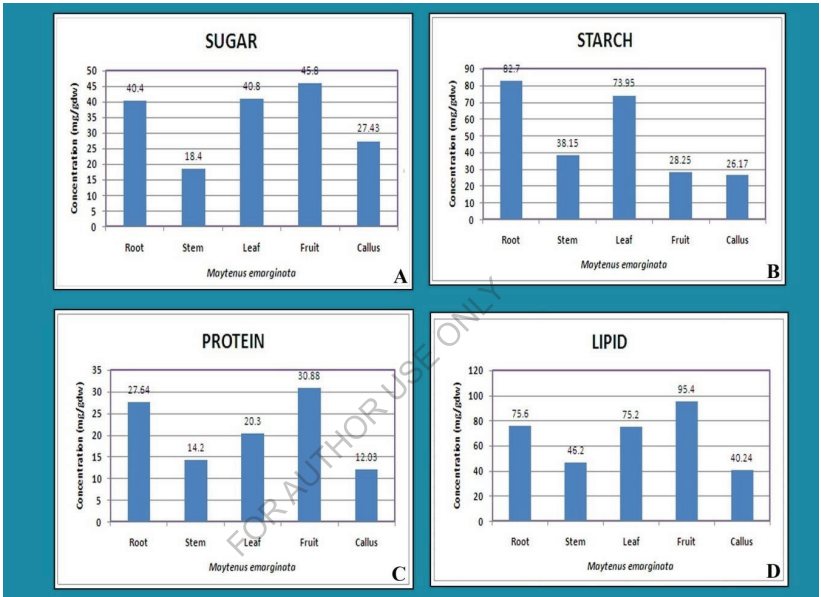
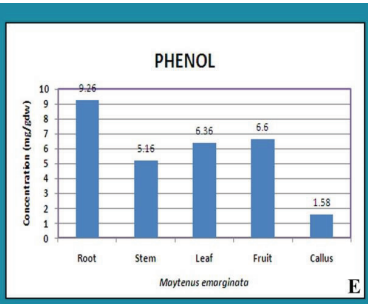
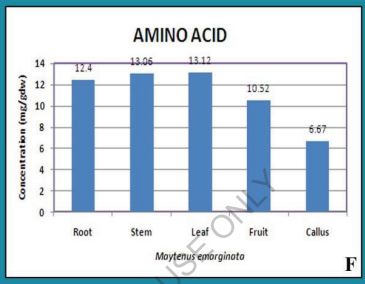


Figure A-D: Estimation of Primary Metabolities in *Maytenus emarginata* (Willd.)

Fig. A : Sugar
Fig. B : Starch
Fig. C : Protein
Fig. D : Lipid





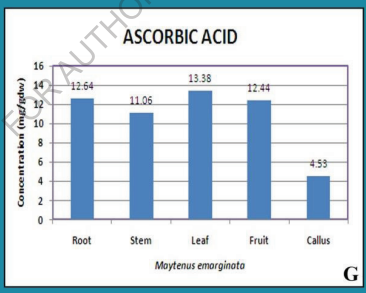


Fig. E-G: Estimation of Primary Metabolities in *Maytenus emarginata* (Willd.).

Fig. E : Phenol
Fig. F : Amino acid
Fig. G : Ascorbic acid

(ii) Secondary metabolites

Flavonoids

The all plant parts (root, stem, leaves, fruits and callus) of *M. emarginata* were studied for flavonoid contents. Each plant sample was extracted with 80% methanol (Subramanian and Nagarajan, 1969).

Presence of two flavonoids kaempferol (Rf 0.86), quercetin (Rf 0.79), have been identified and confirmed in all plant parts and unorganized cultures of *M. emarginata,* whereas kaempferol-7-O-glucoside (Rf 0.83), have been identified and confirmed in only leaves, fruit and callus of *M. emarginata.* Occurrence of luteolin (Rf 0.77), and catechin (Rf 0.36), have been identified and confirmed in all plant parts and unorganized cultures of *M. emarginata.* Identification of the all isolated compound was carried out through thin layer chromatography, Rf value, NMR and IR spectroscopy.

Estimation of flavonoids- *In vivo*

Among all the plant parts of (root, stem, leaves, fruit) of *M. emarginata* stem showed minimum amount of total flavonoids (1.07 mg/gdw) and maximum was found in fruit (1.36 mg/gdw). Increasing amount of total flavonoid content was observed in order as stem (1.07 mg/gdw) < root (1.10 mg/gdw) < leaves (1.13 mg/gdw) < fruit (1.36 mg/gdw).

Estimation of flavonoids- *In vitro*

Eight weeks old callus (maximum GI) was analyzed for identification and estimation of flavonoids in *M. emarginata.*

Occurrence of five flavonoids Kaempferol, Quercetin, Kaempferol-7-O-glucoside, Luteolin and Catechin in *M. emarginata* was also confirmed in their unorganized callus culture. The amount of individual flavonoid as well as total flavonoid content in callus was higher than *in vivo* samples (root, stem, leaves,

fruits). In callus Kaempferol (0.49 mg/gdw) was found in maximum amount and kaempferol-7-O-glucoside (0.19 mg/gdw) in minimum amount.

The result shows the higher levels of flavonoids in the *in vitro* callus of *M. emarginata*. As we know that flavonoid exhibit a number of pharmacological activities, therefore the both plant species are medicinally important and may be used as the source of indigenous medicine.

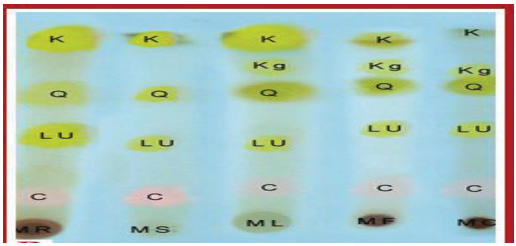


Fig: TLC Chromatogram of isolated flavonoids from *M. emarginata*

Abbreviation:

MR- Maytenus Root, MS- Maytenus Stem, ML- Maytenus leaf, MF- Maytenus Fruit, MC- Maytenus Callus,

K- Kaempferol, Q- Quercetin, Kg- Kaempferol-7-O-glucoside, L-Luteolin, C- Catechin

Table : Chromatographic data and colour reaction of the Flavonoids isolated from *Maytenus emarginata* (Willd.).

Flavonoids	Standards		Recorded Rf values during experiment	Colors in		Colors by chromatogenic sprays				UV max (nm)(MeOH)	m.p (°C)
	Rf value	Color		UV ammonia	I_2 vapours	$FeCl_3$		$AlCl_3$			
						Visible	UV	Visible	UV		
Kampferol	0.86	YG	0.87, 0.86, 0.88, 0.85	BT-YW	YW-BN	BN	BK	YW	YW-GN	253sh, 266sh,294sh,322sh,368sh	312-313
Quercetin	0.79	YG	0.78, 0.79, 0.80	YW	YW-BN	BT-GY	BK	DL-YW	YW-GN	255sh, 269sh,301sh, 374sh	309-311

Luteolin	0.77	YG	0.76, 0.75, 0.77	YW	YW-BN	BN	BK	DL-YW	YW-GN	242sh, 253sh, 267sh, 291sh, 349sh	280 - 320
Kampferol-7-O-glucoside	0.83	Y	0.83, 0.82, 0.80, 0.81	BN	BN	BN	BN	YW	YW	235sh 240sh, 259sh, 374sh, 424sh	317 - 329
Catechin	0.36	P	0.33, 0.36, 0.35	OR	YW-BN	GY	GY-BK	DL-PK	P	256sh,282sh, 296sh, 320sh	294 - 308

Abbreviations: BK = Black , DL = Dull, P= Pink, BN = Brown, GN= Green, OR= Orange, BT = Bright , YW = Yellow

Table: ***In vivo*** **and** ***In vitro*** **flavonoid content** ***Maytenus emarginata*** **(Willd.)**

Flavonoids	***Maytenus emarginata* (Willd.) (mg/gdw)**					
	Root	**Stem**	**Leaves**	**Fruits**	**Callus**	**Total**
Kaempferol	0.38	0.24	0.29	0.27	0.49	1.67
Quercetin	0.28	0.33	0.26	0.32	0.37	1.56
Luteolin	0.24	0.31	0.22	0.39	0.41	1.57
Kaempferol-7-O-glucoside	-	-	0.08	0.14	0.19	0.41
Catechin	0.2	0.19	0.28	0.24	0.32	1.23
Total	1.1	1.07	1.13	1.36	1.78	4.27

Phytosterols

Each of the dried and powdered test samples was defatted in a soxhlet apparatus in petroleum ether (60-80°C) for 24 hrs on a water bath. The residual defatted tissues were dried and then refluxed with 15% (v/v) HCl in 70 % ethanol for 4 hrs (Tomita *et al*., 1970).

Presence of three steroids, lanosterol (Rf 0.92), β-sitosterol (Rf 0.98), and campesterol (Rf 0.290, have been identified and confirmed in all plant parts and unorganized cultures of *M. emarginata,* whereas β-amyrin (Rf 0.98), have been identified and confirmed in only leaves and callus of *M. emarginata.* Occurrence of stigmasterol (Rf 0.88), have been identified and confirmed in all plant parts and unorganized cultures of *M. emarginata.*

Estimation of phytosterols- *In vivo*

Among all the plant parts (root, stem, leaves, and fruit) of *M. emarginata* root showed minimum amount of total steroids (1.99 mg/gdw) and maximum was found in leaves (5.10 mg/gdw). Increasing amount of total steroids content was observed in order as root (1.99 mg/gdw) < fruit (3.99 mg/gdw) < stem (4.53 mg/gdw) < leaves (5.10 mg/gdw).

Estimation of phytosterols- *In vitro*

Eight weeks old callus (maximum GI) was analyzed for identification and estimation of phytosterols in plant *M. emarginata.*

Occurrence of five steroids β-amyrin , β-sitosterol, lanosterol , campesterol and stigmasterol in *M. emarginata* was also confirmed in their unorganized callus culture. The amount of individual steroid as well as total steroid content in callus was higher than *in vivo* samples (root, stem, leaves, and fruits). In callus β-sitosterol (4.98 mg/gdw) was found in maximum amount and β-amyrin (0.15 mg/gdw) in minimum amount.

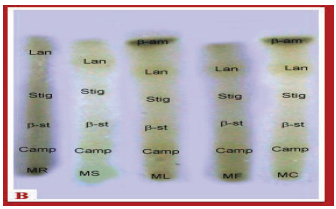


Fig: TLC Chromatogram of isolated Phytosterols from *M. emarginata*

Abbreviation:

MR- Maytenus Root, MS- Maytenus Stem, ML- Maytenus leaf, MF- Maytenus Fruit, MC- Maytenus Callus,

β-am- β-amyrin, β-st- β-sitosterol, Lan- lanosterol , Camp- campesterol, Stg- stigmasterol

Table:- Chromatographic data and color reaction of isolated phytosterols from *Maytenus emarginata* (Willd.) Ding Hou.

Phytosterols	Standards		Recorded Rf values by TLC	Color after heating 50% sulphuric acid in day light				Anisaldehyde reagent	m.p (in °C)	UV max (nm) MeOH
	Rf value	Color		Min.	Initial	Final	In UV			
β-Sitosterol	0.84	DL-RD	0.86, 0.84, 0.83	1.25	GY-PK	PU-BN	DL-RD	PU	135-137	206sh, 268sh, 356sh, 540sh
β-amryin	0.98	BN	0.98, 0.96	1.25	BN	GY	DL-RD	BN	198	240sh, 258sh, 289sh,340sh
Stigmasterol	0.88	GY-BU	0.88, 0.87, 0.86	0.5	PK	GY	BK-BU	PU	143-144	235sh, 270sh, 345sh, 540sh
Lanosterol	0.92	DK-BN	0.94, 0.95, 0.92, 0.93	1.25	PK	PK-BN	DK-BN	PK	131-133	228sh, 245sh, 322sh, 540sh
Campesterol	0.29	DL-BN	0.33, 0.30, 0.28, 0.29	1.25	PK	GY	DL-BN	BU	137-138	210sh, 267sh, 316sh, 540sh

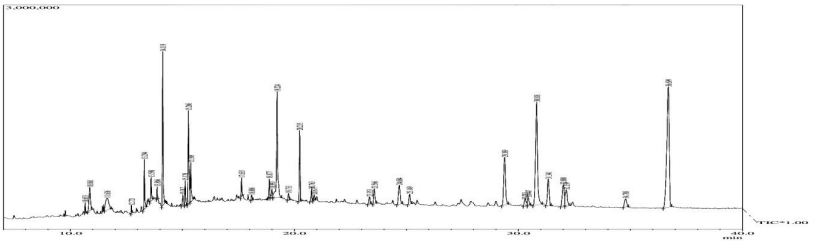
Table:- *In vivo* and *In vitro* sterols content in *Maytenus emarginata* (Willd.) Ding Hou.

Sterols	*Maytenus emarginata* (mg/gdw)					
	Root	Stem	Leaves	Fruits	Callus	Total
β-Sitosterol	1.69	4.19	4.52	3.76	4.98	19.14
β-amryin	-	-	0.08	-	0.15	0.23

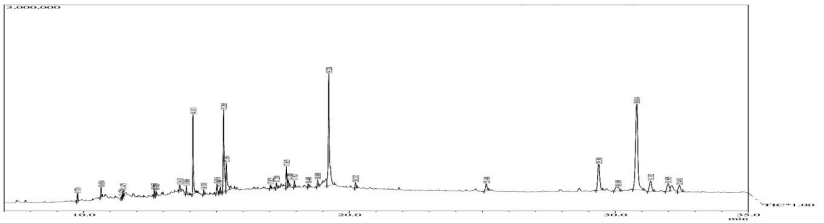
Stigmasterol	0.1	0.13	0.14	0.04	0.18	0.59
Lanosterol	0.05	0.1	0.18	0.04	0.21	0.58
Campesterol	0.15	0.13	0.18	0.15	0.27	0.88
Total	1.99	4.55	5.1	3.99	5.79	21.42

(iii) GC-MS analysis

Gas Chromatography and Mass spectroscopy analysis of compounds were carried out in methanolic extract of (leaves, roots, stems and fruits extract) *M. emarginata*. In the present search it was observed that a variety of compounds have been detected in this plant species including Pentadecanoic acid (Saturated fatty acid), Cholesterol, Stigmasterol, β-sitosterol (Phytosterols), Tridecane, Dodecane (Alkane hydrocabon), Glycerin (alcohol), Myo-inositol (aromatic compound), Evonine (alkaloid), Hexadecen-1ol (terpene alcohol), Hexadecanoic acid (palmitic acid), Phytol (diterpene), Octadecadienoic acid (linoleic acid), Tocopherol (Vitamin-E) and Squalene, Lupeol, Betulin (tri-terpene), Valerenol, alpha-Caryophyllene, Sclareolide (Sesquiterpenes).



(i)



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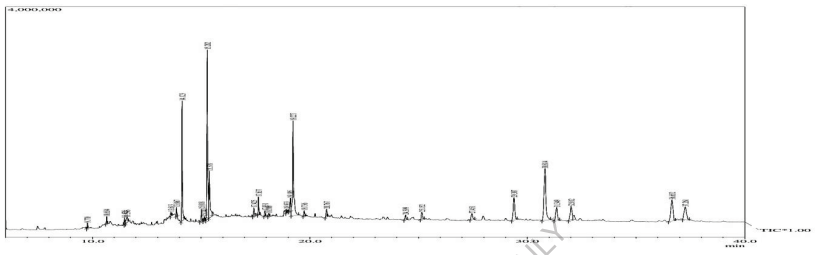

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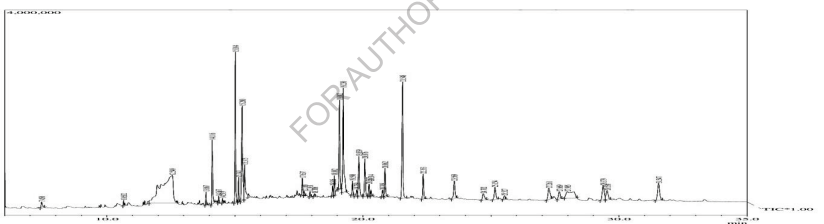


(iv)

Fig: GC/MS spectrogram for the methanolic extract of (i)leaf(ii) root(iii) stem (iv) fruit of *Maytenus emarginata.*

Table-: Activity of phytocomponents identified in the methanolic leaf extract of *Maytenus emarginata.*

S.No	R.T	Name of the Compound	Peak Area %	M.F	M.Wt	Compound Nature

1.	10.653	Pentadecane	0.38	$C_{15}H_{32}$	212	Fragrance agents
2.	10.861	Levoglucosan	2.41	$C_6H_{10}O_5$	162.14	Organic compound
3.	11.636	1,4-Anhydro-d-galactitol	2.73	$C_6H_{12}O_5$	164.14	Sugar alcohol
4.	12.721	Tetradecanoic acid (Myristic acid)	0.34	$C_{14}H_{28}O_2$	228.37	Saturated fatty acid
5.	13.294	Neophytadiene	2.26	$C_{20}H_{38}$	278.51	
6.	13.598	Linoleic acid	1.38	$C_{18}H_{32}O_2$	280.45	unsaturated fatty acid (carboxylic acid)
7.	13.866	Pentadecanoic acid, 14-methyl-, methyl ester	0.43	$C_{17}H_{34}O_2$	270	fatty acid
8.	14.119	n-Hexadecanoic acid (Palmitic acid)	7.16	$C_{16}H_{32}O_2$	256	fatty acid
9.	15.017	Palmitoleic acid methyl ester	0.72	$C_{17}H_{32}O_2$	268.43	unsaturated fatty acid
10.	15.120	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	1.26	$C_{20}H_{40}O$	296.53	Diterpene alcohol
11.	15.266	9-Octadecenoic acid (Oleic Acid)	6.08	$C_{18}H_{34}O_2$	282	unsaturated fatty acid
12.	15.369	Octadecanoic acid (Stearic acid)	2.18	$C_{18}H_{36}O_2$	284	Saturated fatty acid

13.	17.635	Palmitic acid .beta.-monoglyceride	0.80	$C_{19}H_{38}O_4$	330	Fatty acid derivative
14.	18.086	n-Hexadecane sulfonyl chloride	0.34	$C_{16}H_{33}ClO_2S$	324	
15.	18.877	N-Hexacosane	0.83	$C_{26}H_{54}$	366.71	Fragrance agent
16.	18.993	Octadecanedioic acid	0.70	$C_{18}H_{34}O_4$	314	Fatty acid
17.	19.224	Alpha.-Monostearin	8.17	$C_{21}H_{42}O_4$	358	Saturated fatty acid
18.	19.733	1-Hentetracontanol	0.45	$C_{41}H_{84}O$	592	Terpenoid
19.	20.235	Squalene	3.52	$C_{30}H_{50}$	410.72	Triterpene
20.	20.763	Nonacosane	0.82	$C_{29}H_{60}$	408	Hydrocarbon
21.	20.877	9-Tricosene, (Z)	0.40	$C_{23}H_{46}$	322	
22.	23.353	gamma.-Tocopherol	0.69	$C_{28}H_{48}O_2$	416.68	vitamin E
23.	23.566	1-Triacontanol	1.29	$C_{30}H_{62}O$	438.81	Fatty alcohol
24.	24.684	dl-.alpha.-Tocopherol	2.19	$C_{29}H_{50}O_2$	430	vitamin E
25.	25.149	Cholesterol	0.94	$C_{27}H_{46}O$	386.65	Phytosterol
26.	29.389	β-Sitosterol	5.95	$C_{29}H_{50}O$	414	Phytosterol
27.	30.283	Lupenyl acetate	0.93	$C_{32}H_{52}O_2$	468	Tri- terpenes
28.	30.447	Ursodeoxycholic acid (Ursodiol)	0.89	$C_{24}H_{40}O_4$	392	Secondary bile acids
29.	30.818	Methyl commate A	14.21	$C_{32}H_{52}O_4$	500	Triterpenes glycoside

30.	31.341	Lupeol	3.12	$C_{30}H_{50}O$	426.73	Triterpenoid
31.	32.008	Betulin	2.93	$C_{30}H_{50}O_2$	442.72	Triterpene
32.	32.139	alpha.-Amyrin acetate	2.32	$C_{32}H_{52}O_2$	468	Phytosterol
33.	34.788	Sclareolide	1.28	$C_{16}H_{26}O_2$	250.38	Sesquiterpene
34.	36.699	Friedelin	19.91	$C_{30}H_{50}O$	426.73	Pentacyclic triterpene

Abbreviations: RT = Retention time, MF = Molecular formula, M Wt = Molecular weight

Table-: Activity of phytocomponents identified in the methanolic root extract of *Maytenus emarginata.*

S.No	R.T	Name of the Compound	Peak Area %	M.F	M.Wt	Compound Nature
1.	9.770	Tridecane	0.68	$C_{13}H_{28}$	184.35	Alkane hydrocarbon
2.	10.654	Pentadecane	0.92	$C_{15}H_{32}$	212	Fragrance agents
3.	11.425	1-hexadecanol	0.18	$C_{16}H_{34}O$	242	Fatty alcohol
4.	11.475	Trichloroacetic acid, dodecyl ester	0.56	$C_{14}H_{25}C_{l3}O_2$	330	Fatty acid
5.	12.653	1,2-Dimethyl-3,5-divinylcyclohexane	0.52	$C_{12}H_{20}$	164	
6.	12.725	Tetradecanoic acid (Myristic acid)	0.46	$C_{14}H_{28}O_2$	228.37	Saturated fatty acid

7.	13.613	Diisobutyl Phthalate	0.74	$C_{16}H_{22}O_4$	278.35	Plastisicizer
8.	13.866	Pentadecanoic acid, 14-methyl-, methyl ester	0.67	$C_{17}H_{34}O_2$	270	Fatty acid
9.	14.113	n-Hexadecanoic acid (Palmitic acid)	8.16	$C_{16}H_{32}O_2$	256	Fatty acid
10.	14.518	Eicosanoic acid, methyl ester	0.39	$C_{21}H_{42}O_2$	326	Fatty acid
11.	15.016	9-Hexadecenoic acid, methyl ester, (Z)-	1.12	$C_{17}H_{32}O_2$	268	Fatty acid
12.	15.139	Octadecanoic acid, methyl ester	0.55	$C_{19}H_{38}O_2$	298	Fatty acid
13.	15.265	9-Octadecenoic acid (Z)- (Oleic Acid)	11.93	$C_{18}H_{34}O_2$	282	Unsaturated fatty acid
14.	15.367	Octadecanoic acid (Stearic acid)	4.32	$C_{18}H_{36}O_2$	284	Saturated fatty acid
15.	17.025	Hexadecanal	0.35	$C_{16}H_{32}O$	240	Aldehyde
16.	17.250	2-Ethyl-9,10-anthracenediol	0.78	$C_{16}H_{14}O_2$	238	Unsaturated fatty acid
17.	17.635	Palmitic acid .beta.-monoglyceride	2.52	$C_{19}H_{38}O_4$	330	Fatty acid derivative
18.	17.700	n-Octadecanal	0.51	$C_{18}H_{36}O$	268	Aldehyde
19.	17.927	Diisooctyl phthalate	0.62	$C_{24}H_{38}O_4$	390.56	plastisicizer
20.	18.808	Cyclocarbosilane	0.80	$C_3H_{12}Si_3$	132	

21.	19.224	alpha.-Monostearin	17.63	$C_{21}H_{42}O_4$	358	
22.	20.232	Squalene	0.70	$C_{30}H_{50}$	410.72	Triterpene
23.	25.148	Cholesterol	1.50	$C_{30}H_{50}$	410.72	Phytosterol
24.	29.381	B-Sitosterol	7.11	$C_{29}H_{50}O$	414.71	Phytosterol
25.	30.109	Methyl commate C	1.84	$C_{31}H_{50}O_4$	486	Triterpenes glycoside
26.	30.814	Methyl commate A	26.56	$C_{32}H_{52}O_4$	500	Triterpenes glycoside
27.	31.332	Lupeol	3.18	$C_{30}H_{50}O$	426.73	Triterpenoid
28.	31.995	Farnesyl bromide	2.42	$C_{15}H_{25}Br$	284	
29.	32.418	Longifolenaldehyde	1.91	$C_{15}H_{24}O$	220.35	

Abbreviations: RT = Retention time, MF = Molecular formula, M Wt = Molecular weight

Table-: Activity of phytocomponents identified in the methanolic stem extract of *Maytenus emarginata.*

S.No	R.T	Name of the Compound	Peak Area %	M.F	M.Wt	Compound Nature
1.	9.770	Tridecane	0.37	$C_{13}H_{28}$	184.35	Alkane hydrocarbon
2.	10.654	Pentadecane	0.56	$C_{15}H_{32}$	212	Fragrance agents
3.	11.475	Trichloroacetic acid, dodecyl ester	0.48	$C_{14}H_{25}C_{13}O_2$	330	Fatty acid
4.	11.596	Sorbitol	1.18	$C_6H_{14}O_6$	182.17	Sugar alcohol

5.	13.613	Diisobutyl Phthalate	0.37	$C_{16}H_{22}O_4$	278.35	
6.	13.867	Pentadecanoic acid, 14-methyl-, methyl ester	0.72	$C_{17}H_{34}O_2$	270	Fatty acid
7.	14.123	n-Hexadecanoic acid (Palmitic acid)	10.48	$C_{16}H_{32}O_2$	256	Saturated fatty acid
8.	15.018	9-Octadecenoic acid (Oleic acid)	1.07	$C_{18}H_{34}O_2$	282.46	Unsaturated fatty acid
9.	15.141	Octadecanoic acid, methyl ester	0.43	$C_{19}H_{38}O_2$	298	Unsaturated fatty acid
10.	15.282	9-Octadecenoic acid (Z)- (Oleic acid)	19.86	$C_{18}H_{34}O_2$	282	Unsaturated fatty acid
11.	15.378	Octadecanoic acid (Stearic acid)	5.87	$C_{18}H_{36}O_2$	284	Saturated fatty acid
12.	17.425	1-Heneicosyl formate	0.93	$C_{22}H_{44}O_2$	340	Volatile and semivolatile organic compound
13.	17.637	Palmitic acid .beta.-monoglyceride	1.65	$C_{19}H_{38}O_4$	330	Unsaturated fatty acid
14.	17.931	1,2-Benzenedicarboxylic acid, dioctyl ester	0.46	$C_{24}H_{38}O_4$	390	Unsaturated fatty acid
15.	18.106	Pentadecane, 8-hexyl-	0.34	$C_{21}H_{44}$	296	Fatty acid
16.	18.933	1-Eicosanol	0.35	$C_{20}H_{42}O$	298	Fatty acid alcohol
17.	19.105	2-[5-(2-Methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]-	1.80	$C_{17}H_{13}N_3O_2$	291	Steroid

		phenol				
18.	19.227	alpha.-Monostearin	13.47	$C_{21}H_{42}O_4$	358	
19.	19.736	1-Hentetracontanol	0.71	$C_{41}H_{84}O$	592	Triterpene
20.	20.767	n-Tetratetracontane	0.94	$C_{44}H_{90}$	619.19	Triterpenoid
21.	24.399	4,6-Cholestadien-3.beta.-ol	0.65	$C_{27}H_{44}O$	384.64	Steroid
22.	25.152	Cholesterol	1.18	$C_{30}H_{50}$	410.72	Phytosterol
23.	27.451	n-Tetracosano (Lignoceric alcohol)	1.25	$C_{24}H_{50}O$	354	Fatty acid
24.	29.387	Beta.-Sitosterol	4.98	$C_{29}H_{50}O$	414	Phytosterol
25.	30.814	Methyl commate A	12.95	$C_{32}H_{52}O_4$	500	Triterpenes glycoside
26.	31.349	Lupeol	2.94	$C_{30}H_{50}O$	426.73	Triterpenoid
27.	32.012	Betulin	3.72	$C_{30}H_{50}O_2$	442.72	Triterpene
28.	36.652	Friedelin	6.15	$C_{30}H_{50}O$	426	Triterpene
29.	37.261	Alpha.-Bisabolol	4.15	$C_{15}H_{26}O$	222	Unsaturated sesquiterpene alcohol

Abbreviations: RT = Retention time, MF = Molecular formula, M Wt = Molecular weight

Table: Activity of phytocomponents identified in the methanolic fruit extract of *Maytenus emarginata.*

S.No	R.T	Name of the Compound	Peak Area	M.F	M.Wt	Compound Nature

			%			
1.	7.450	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.49	$C_6H_8O_4$	144	
2.	10.652	Pentadecane	0.22	$C_{15}H_{32}$	212	Fragrance agent
3.	12.549	1,2,3,4,5-Cyclohexanepentol	23.17	$C_6H_{12}O_5$	164	
4.	13.867	Pentadecanoic acid, 14-methyl-, methyl ester	0.51	$C_{17}H_{34}O_2$	270	Fatty acid
5.	14.116	n-Hexadecanoic acid (Palmitic acid)	3.22	$C_{16}H_{32}O_2$	256	Fatty acid
6.	14.367	6,6-Dimethylcycloocta-2,4-dienone	0.28	$C_{10}H_{14}O$	150	
7.	14.519	Eicosanoic acid, methyl ester	0.19	$C_{21}H_{42}O_2$	326	Fatty acid
8.	15.014	8-Octadecenoic acid, methyl ester	7.12	$C_{19}H_{36}O_2$	296	Fatty acid
9.	15.141	Octadecanoic acid, methyl ester	0.99	$C_{19}H_{38}O_2$	298	Fatty acid
10.	15.280	9-Octadecenoic acid (Oleic Acid)	7.75	$C_{18}H_{34}O_2$	282	Unsaturated fatty acid
11.	15.375	Octadecanoic acid (Stearic acid)	2.72	$C_{18}H_{36}O_2$	284	Saturated fatty acid
12.	17.637	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.97	$C_{19}H_{38}O_4$	330	Fatty acid
13.	17.708	n-Octadecanal	0.28	$C_{18}H_{36}O$	268	Unsaturated

						fatty acid
14.	17.931	1,2-Benzenedicarboxylic acid, dioctyl ester	0.28	$C_{24}H_{38}O_4$	390	Fatty acid
15.	18.106	Pentadecane, 8-hexyl-	0.17	$C_{21}H_{44}$	296	Fatty acid
16.	18.816	1H-Indole-3-ethanamine	0.62	$C_{10}H_{12}N_2$	160	
17.	18.882	n-Heneicosane	1.10	$C_{21}H_{44}$	296	
18.	19.082	Valerenol	5.51	$C_{15}H_{24}O$	220	Sesquiterpenoid
19.	19.230	Alpha.-Monostearin	7.80	$C_{21}H_{42}O_4$	358	Fatty acid derivative
20.	19.590	1,4,4,7a-Tetramethyl-2,4,5,6,7,7a-hexahydro-1H-indene-1,7-diol	0.82	$C_{13}H_{22}O_2$	210	Unsaturated fatty acid
21.	19.759	N-Nonadecane	0.48	$C_{19}H_{40}$	268	Saturated aliphatic hydrocarbon
22.	19.839	Germacrene A	2.89	$C_{15}H_{24}$	204.3	Volatile organic hydrocarbons (sesquiterpene)
23.	20.076	Seychellene	2.42	$C_{15}H_{24}$	204.3	
24.	20.237	Squalene	0.70	$C_{30}H_{50}$	410.7	Triterpene
25.	20.314	3-Amino-6-methyl-6,7-dihydro-9H-5-oxa-9-azabenzocyclohepten-8-one	0.38	$C_{10}H_{12}N_2O_2$	192.2	Steroid organic compound
26.	20.768	Nonacosane	0.40	$C_{29}H_{60}$	408	Hydrocarbon
27.	20.862	Perillaldehyde	2.05	$C_{10}H_{14}O$	150.2	Monoterpenoid
28.	21.548	Isovellerdiol	8.56	$C_{15}H_{24}O_2$	236	Sesquiterpenes

29.	22.353	Andrographolide	1.84	$C_{20}H_{30}O_5$	350.4	diterpenoid
30.	23.569	1-Triacontanol	1.77	$C_{30}H_{62}O$	438.8	Fatty alcohol
31.	24.702	A-Tocopherol	0.80	$C_{29}H_{50}O_2$	430.7	Vitamin- E
32.	25.154	Cholesterol	1.40	$C_{30}H_{50}$	410.7	Steroid
33.	25.527	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-(Widdrol)	0.35	$C_{15}H_{26}O$	222.3	Odorous compound
34.	27.261	alpha.-Tocopherol	1.58	$C_{29}H_{50}O_2$	430	Vitamin- E
35.	27.669	Trilostane	0.87	$C_{20}H_{27}NO$	329.4	3β-hydroxysteroid dehydrogenase inhibitor
36.	27.985	Evonine	3.36	$C_{36}H_{43}NO_{17}$	761	Alkaloid
37.	29.379	B-Sitosterol	2.11	$C_{29}H_{50}O$	414.7	Phytosterol
38.	29.538	1,4,8-Cycloundecatriene, 2,6,6,9-tetramethyl-, (E,E,E)- (alpha- Caryophyllene)	1.36	$C_{15}H_{24}$	204.35	sesquiterpenes
39.	31.547	Cycloartenol	2.48	$C_{30}H_{50}O$	426.72	Phytostanol

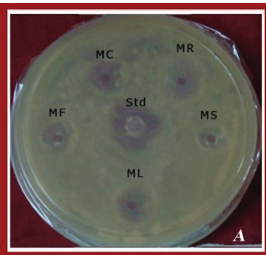
Abbreviations: RT = Retention time, MF = Molecular formula, M Wt = Molecular weight

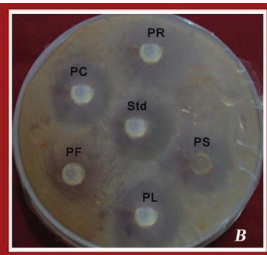
(III) BIOACTIVITIES

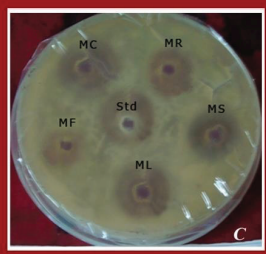
(i) Antimicrobial activity

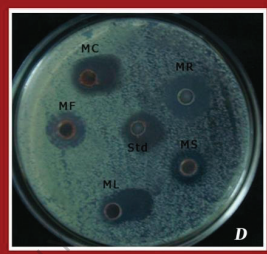
In the present investigation, the inhibitory effect of crude methanolic extracts of different plant parts from *M. emarginata* were evaluated against both fungicidal and bacterial strains (gram positive and gram negative). *In vitro* and *in vivo* antimicrobial activity was determined using Agar well diffusion method and Micro dilution method.

In *Maytenus emarginata* the maximum antimicrobial activity of root extract was observed against *E. coli and F. oxisporum* while in leaf extract maximum antimicrobial activity was observed against *E. cloacae, P. aeruginosa, A. niger* and *A. flavus*. However, Callus extracts showed mild to moderate inhibitory effects against *S. aureus, B. cereus and A. solani*. Besides these, the maximum antimicrobial activity of *Maytenus* fruit extract was observed only against *R. stolonifer*. Thus, the result of the present study gives clue that the extracts of the both plant species can be regarded as a potential source for antimicrobial drugs.









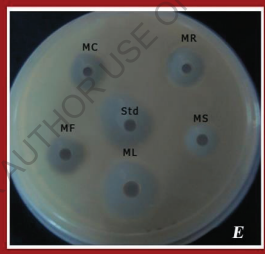


Fig. A-E : Antimicrobial activity of *Maytenus emarginata* (Willd.) against various bacteria.

Fig. A : Methanolic extract of different plant parts of *M. emarginata* against *E. coli.*
Fig. B : Methanolic extract of different plant parts of *M. emarginata* against *E. cloacae.*
Fig. C : Methanolic extract of different plant parts of *M. emarginata* against *S. aureus.*
Fig. D : Methanolic extract of different plant parts of *M. emarginata* against *B. cereus.*
Fig. E : Methanolic extract of different plant parts of *M. emarginata* against *P. aeruginosa.*

ABBREVIATIONS

Std = Standard	ML = Maytenus Leaf
MR = Maytenus Root	MF = Maytenus Fruit
MSt = Maytenus Stem	MC = Maytenus Callus

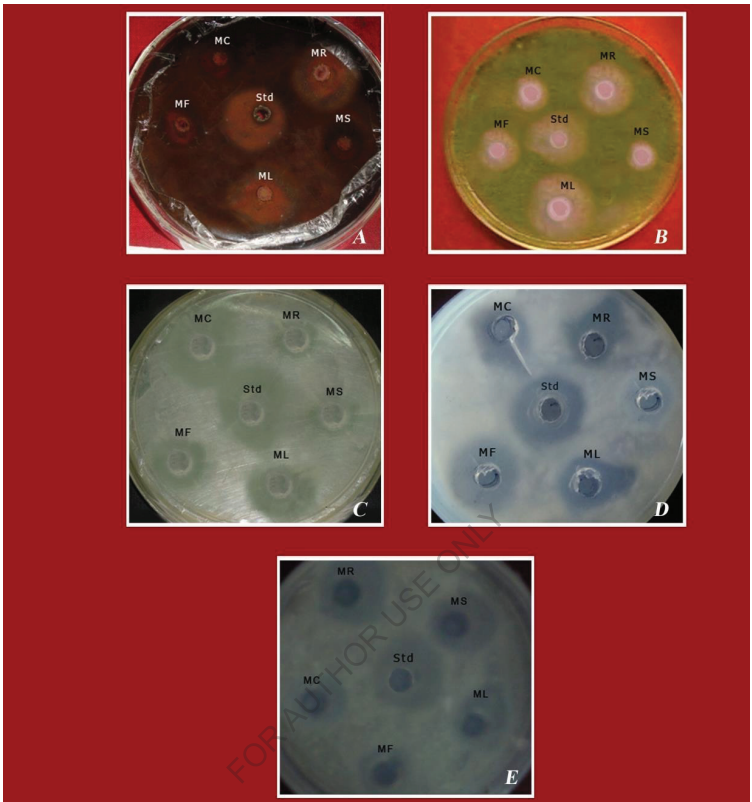


Fig. A-E : Antimicrobial activity of *Maytenus emarginata* (Willd.) against various fungi.

Fig. A : Methanolic extract of different plant parts of *M. emarginata* against *A. niger.*
Fig. B : Methanolic extract of different plant parts of *M. emarginata* against *A. flavus.*
Fig. C : Methanolic extract of different plant parts of *M. emarginata* against *A. solani.*
Fig. D : Methanolic extract of different plant parts of *M. emarginata* against *R. stolonifer.*
Fig. E : Methanolic extract of different plant parts of *M. emarginata* against *F. oxisporum*

ABBREVIATIONS

Std = Standard	ML = Maytenus Leaf
MR = Maytenus Root	MF = Maytenus Fruit
MSt = Maytenus Stem	MC = Maytenus Callus

Table:- Inhibition zone diameters in methanolic extract of different plant parts of *Maytenus emarginata* were measured by agar well diffusion method (in mm).

Test Organisms		*Maytenus emarginata* (Willd.)					
Bacteria		**IZ / AI**	**Root**	**Stem**	**Leaf**	**Fruit**	**Callus**
1	***E.coli***	**IZ**	19.2 ± 0.32	10.8 ± 0.91	18.3 ± 0.71	12.09 ± 0.49	14.9 ± 0.55
		AI	0.786	0.442	0.75	0.495	0.61
2	***E.cloacae***	**IZ**	13.2 ± 0.32	9.68 ± 0.55	20.3 ± 0.92	9.8 ± 0.69	15.8 ± 0.45
		AI	0.692	0.507	1.064	0.513	0.828
3	***S.aureus***	**IZ**	12.8 ± 0.75	10.09 ± 0.49	14.2 ± 0.56	8.6 ± 0.59	17.5 ± 0.49
		AI	1.013	0.796	1.124	0.68	1.385
4	***B. cereus***	**IZ**	16.01 ± 0.63	15.02 ± 0.81	19.9 ± 0.59	15.4 ± 0.89	20.08 ± 0.29
		AI	0.743	0.697	0.924	0.715	0.933
5	***P. aeruginosa***	**IZ**	20.3 ± 0.92	17.8 ± 0.71	22.4 ± 0.86	18.9 ± 0.11	19.2 ± 0.42
		AI	0.989	0.867	1.092	0.921	0.936
Fungi		**IZ / AI**	**Root**	**Stem**	**Leaf**	**Fruit**	**Callus**
1	***A. niger***	**IZ**	12.8 ± 0.79	9.2 ± 0.55	15.6 ± 0.53	10.3 ± 0.63	9.9 ± 0.35
		AI	0.749	0.538	0.913	0.603	0.579
2	***A. flavus***	**IZ**	14.2 ± 0.52	8.9 ± 0.75	18.3 ± 0.29	11.9 ± 0.42	10.4 ± 0.71
		AI	0.805	0.504	1.038	0.674	0.589
3	***A. solani***	**IZ**	10.3 ± 0.13	8.2 ± 0.41	14.3 ± 0.77	12.9 ± 0.64	16.8 ± 0.39
		AI	1.17	0.931	1.625	1.465	1.909
4	***R. stolonifer***	**IZ**	9.5 ± 0.80	9.4 ± 0.72	12.5 ± 0.65	13.4 ± 0.51	8.6 ± 0.52
		AI	0.619	0.613	0.815	0.874	0.56
5	***F. oxisporum***	**IZ**	20.1 ± 0.59	18.2 ± 0.65	14.8 ± 0.29	10.6 ± 0.71	13.8 ± 0.42
		AI	0.928	0.841	0.683	0.489	0.637

Table:- MIC values obtained in methanolic extract of different plant parts of *Maytenus emarginata* were measured by micro dilution method (in µg/ml).

Test Organisms		*Maytenus emarginata* (Willd.)						
Bacteria		**MIC / MBC**	**Standard**	**Root**	**Stem**	**Leaf**	**Fruit**	**Callus**
1	***E.coli***	**MIC**	20.1	30.2	61.3	41.9	58.9	45.8
		MBC	40.3	62.8	109.6	79.6	106.3	90.1
2	***E.cloacae***	**MIC**	33.2	37.7	54.2	31.8	49.8	30.7
		MBC	60.9	74.1	104.6	62.9	90.2	60.6
3	***S.aureus***	**MIC**	20.6	32.8	48.9	22.9	60.9	18.3
		MBC	40.1	67.3	96.2	46.8	119.6	38.9
4	***B. cereus***	**MIC**	23.5	34.3	48.3	26.9	39.2	22.4

		MBC	64.9	66.8	98.7	56.3	78.1	46.3
		MIC	22.01	37.9	52.1	20.1	49.3	43.5
5	***P. aeruginosa***	**MBC**	45.3	72.01	103	41.2	97.8	86.4
Fungi		**MIC / MFC**	**Standard**	**Root**	**Stem**	**Leaf**	**Fruit**	**Callus**
		MIC	9.3	21.9	52.1	14.4	30.5	35.4
1	***A. niger***	**MFC**	20.4	41.8	100	38.6	62.8	70.9
		MIC	10	20.1	42.4	14.2	28.1	35.3
2	***A. flavus***	**MFC**	20.3	40.3	85.2	29.3	57.2	76.1
		MIC	36.3	51.2	54.8	42.5	48.6	30.2
3	***A. solani***	**MFC**	72.4	102	112.5	86.3	96.2	62.1
		MIC	11.7	21.7	32.5	18.8	10.9	39.9
4	***R. stolonifer***	**MFC**	23.9	44.8	65.6	38.1	22.4	81.2
		MIC	30.7	29.8	34.8	40.2	49.7	43.3
5	***F. oxisporum***	**MFC**	57.6	56.1	68.3	83.6	98.2	86.6

Abbreviations:- MIC = Minimum inhibition concentration,
MBC= Minimum bacterial concentration
MFC= minimum fungicidal concentration

(ii) Antioxidant activity

The antioxidant activity of methanolic extracts of different plant parts were investigated using DPPH. During the present research study, it was found that all plant extracts have shown remarkable antioxidant activities. However, IC_{50} values of different plant parts have also been estimated to exhibit their antioxidant activities and compared with standard ascorbic acid. it is found that in *Maytenus emarginata* IC_{50} 49.32µg/ml of root extract indicates the maximum antioxidant effect.

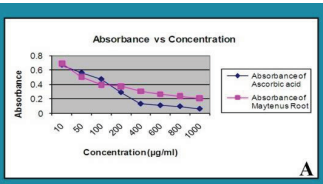


Fig.A: DPPH scavenger assay of the root extract of *Maytenus emarginata* compared with standard ascorbic acid.

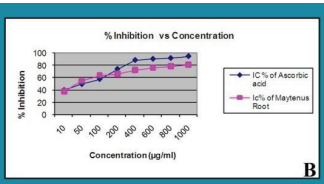


Fig.B: Evalution of IC_{50} of the root extract of *Maytenus emarginata* and standard ascorbic acid.

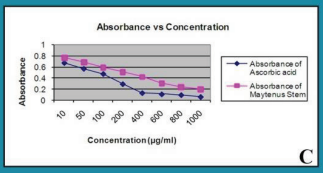


Fig.C: DPPH scavenger assay of the stem extract of *Maytenus emarginata* compared with standard ascorbic acid.

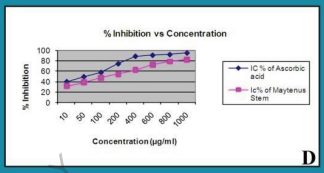


Fig.D: Evalution of IC_{50} of the stem extract of *Maytenus emarginata* and standard ascorbic acid.

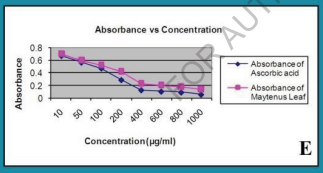


Fig.E: DPPH scavenger assay of the leaf extract of *Maytenus emarginata* compared with standard ascorbic acid.

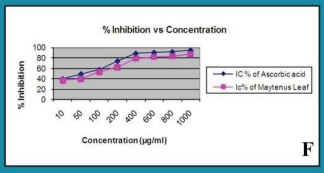


Fig.F: Evalution of IC_{50} of the leaf extract of *Maytenus emarginata* and standard ascorbic acid.

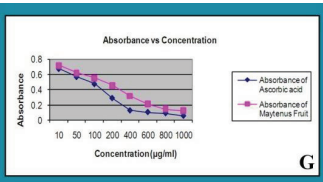


Fig.G: DPPH scavenger assay of the fruit extract of *Maytenus emarginata* with standard.

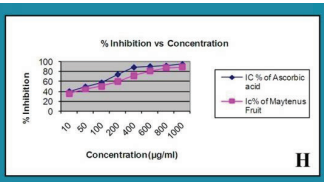


Fig.H: Evalution of IC_{50} of the fruit extract of *Maytenus emarginata* and standard ascorbic acid.

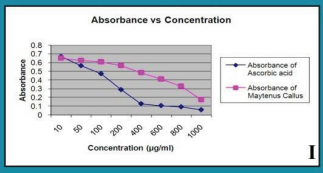


Fig.I: DPPH scavenger assay of the callus extract of *Maytenus emarginata* compared with standard ascorbic acid.

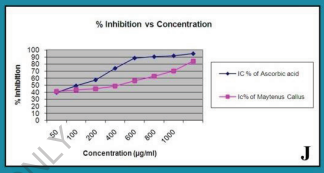


Fig.J: Evalution of IC_{50} of the callus extract of *Maytenus emarginata* and standard ascorbic acid.

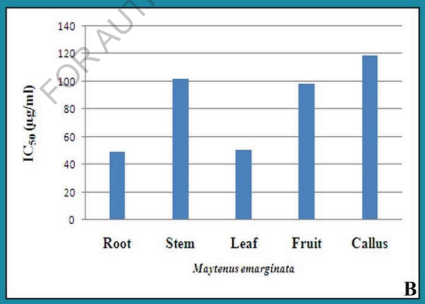


Fig. B: Values of IC_{50}% in different plant parts of *Maytenus emarginata.*