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Organoleptic, Microscopic Evaluation and Invitro Propagation of Mollugo Oppositifolia L.

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ABSTRACT

Mollugo oppositifolia L. commonly called as carpetweed, is a prostrate herb, belonging to family Molluginaceae. It is used in the treatment of skin disease, piles, leucoderma, urinary infections, liver problem and also used as antioxidant. Organoleptic and Microscopic studies are useful to establish the botanical identity for the valuable crude drugs, which forms the basis for the identification and determination of adulterants. The root of Mollugo oppositifolia is circular in outline and shows a narrow cork made up of 3-4 layers of tangentially elongated and radially arranged suberized cells, fibres and uni to bi-seriate thin walled xylem rays. The transverse section of the stem is wavy in outline, glandular trichomes with multi-cellular stalk and uni to bicellular head. The leaf has rosettes and prisms of calcium oxalate scattered throughout lamina. These characters help in identifying the adulterants from the original raw drugs supplied to Pharmaceutical companies. Further to overcome the constrains in cultivation, collection and to meet their high demand in herbal drug industries, a rapid in vitro propagation of this valuable medicinal plant was carried out. BAP (2 mg/l) produced multiple shoots with 86% of response after 8 weeks of inoculation. Highest callus was obtained from the stem explants inoculated in NAA 3mg/l.

KEY WORDS

Mollugo oppositifolia, Organoleptic evaluation, Microscopic evaluation, Adulteration, Invitro propagation.

INTRODUCTION

Medicinal plants have always been considered a healthy source of life for people. Therapeutic properties of medicinal plants are very useful in healing various diseases and the advantage of these medicinal plants is being 100% natural. Farnsworth and Soejarto (1990) have estimated that about 35,000 - 75,000 species out of the 250,000 flowering plants in the world, are used for medicinal purpose. Herbal drug industries mainly depends on wild plants as a source of raw drugs. The raw materials are often subjected to adulteration and substitution when procured from market (Handa, 2004). Organoleptic (Morphological) and Microscopic evaluation play a very important role in drug evaluation.

Evaluation of a drug is confirmation of its identity and determination of its quality, purity, detection of nature of adulteration and standardization of the crude drug. The evaluation of a crude drug

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is necessary because of biochemical variation in the drug, deterioration due to treatment and storage and substitution and adulteration as a result of carelessness, ignorance or fraud. Evaluation of the drug can be confirmed by comparing the crude drug sample with an already established crude drug or by evaluating the crude substance according to the Official Literature available about that drug in pharmacopoeias (Anonymous., 2001).

Organoleptic evaluation means to study the subject i.e. crude drug with the help of five senses, general morphological features and physical characteristics like shape, structure, color, smell, taste, sound of fracture etc, can be the elements of organoleptic evaluation. Microscopic evaluation or examination means the study of histological and cytological features of the organized drug. Microscopic characters like types of cells, tissues present, cell composition like starch, calcium oxalate, and mucilage, special plant structures like stomatal apparatus or trichomes has to be studied.

Excessive extraction of medicinal plant resources for use in the herbal industries have resulted in ruthless destruction of natural population of medicinal plants. The tissue culture methods of plant propagation, known as Micropropagation, utilizes the culture of apical shoots, axillary buds and meristems on suitable nutrient medium. Medicinal plants produce a vast array of secondary metabolites (Croteau, 2000).

Mollugo oppositifolia L. belongs to the family Molluginaceae. It is commonly known as carpet weed. It is an erect slender, much branched annual herb, up to 30 cm. high, commonly found in dry as well as moist areas. It is also having numerous applications in traditional medicine as stomachic, aperients, antiseptic, emmenagogue and is also used in poultices for sore legs. An infusion of the plant is given to women to promote the menstrual discharge. Leaves are bitter and antiperiodic, they are warmed after smearing with oil and applied to the ear to relieve earache. *M. oppositifolia* is used in the treatment of skin disease, increase appetite, cures kapha, piles, leucoderma, tonic to intestine, urinary infections, fever, cough, liver problem and also used as antioxidant due to its excellent properties and potent phytoconstituents activities like free radical scavenging and antioxidant activities, hepatoprotective effect, antiprotozoal activity, immunomodulating activity of *M. oppositifolia* has been reported (Anonymous,1982; Chopra *et al* .,1956). In the herbal industry this plant is used as an adulterant for *Hedyotis corymbosa* which belongs to the family Rubiaceae.

The aim of the study is to examine the organoleptic characters and microscopic characters of the plant *Mollugo oppositifolia* and list out the characteristic characters by which the plant can be identified easily. The objective of the study is that these characters can be used by the Pharmaceuticals companies. Whenever the dried *Mollugo oppositifolia* plant is received from the whole sale sellers the Pharmaceutical companies can use these characters as identification tools to confirm the purity of the drugs. The coast of the crude drugs is decided based on the purity of the herbal drugs received, pure the crude drug is, higher the cost they fetch. *In vitro* studies aim at standardizing a protocol for shoot tip and callus culture.

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MATERIALS AND METHODS

Mollugo oppostifolia L. which belongs to Molluginaceae was collected and identified with the help of Flora of Presidency of Madras (Gamble, 1957). The plants were collected and studied for various morphological characters. The plant parts were examined and technical descriptions were written. Photographs were taken using Nikon camera.

Mature leaf, stem and root of *Mollugo oppositifolia* were collected for anatomical studies and preserved in Formalin Acetic Acid Alcohol mixture (FAA) (Johansen, 1940). The standard Microtechnique with some modifications in the procedure was followed for taking anatomical sections (Sass, 1958 & Johansen, 1940). Cross sections of leaf, stem and root were stained with Toludine blue (O'Brien *& al*., 1964) and carefully examined. The measurements of all the cross sections were made using ocular micrometer. Photographs of all the anatomy preparations like lamina, midrib, stem and root were taken using Nikon Digital camera.

Young, healthy and disease free explants were used for *in vitro* studies. The explants used were axillary bud and stem. The MS basal medium formulation described by Murashige and Skoog,(1962) was selected as the optimal culture medium. Healthy and young explants, nodal axillary buds and stem of about 1-1.5 cm were selected for sterilization. The excised explants were thoroughly washed in running tap water for about 15-20 minutes and in 5 % (w/v) detergent solution of Tween 20 for 10 minutes. After thorough rinsing in sterile distilled water the explants were surface sterilised with 70% ethanol for 30 seconds and then in 0.02%(w/v) aqueous $HgCl_2$ for 3-5 minutes under aseptic condition. Sterilized explants were transferred to the inoculation chamber, and washed with double distilled sterile water for at least 7 times to remove any trace of surface sterilizing chemicals present on the surface of the explants. The *in vitro* cultures were maintained at 25±2°C and 3000 Lux. Illumination comprising a 16 hour photoperiod provided by cool fluorescent light and with a relative humidity of 50±20%.

RESULTS AND DISCUSSION

MORPHOLOGY

The plant is a prostrate shrub. The stem is glabrous and prostrate. Leaves are simple and arranged in whorled. The shape of the leaves are spathulate and margin is entire. Infloresence is axillary fascicle and flowers are white. Sepals five in number and corolla absent, ten stamens are arranged in two whorls. The outer whorl is fertile and inner whorl is staminode and it alters with the sepals. The anthers are dithecous and basifixed. Gynoecium is tricarpellary, syncarpous and trilocular. The ovules are in axile placentation. The style is simple and stigma trifid. The flowering and fruiting season is throughout the year (Fig. 1 a, b).

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FIGURE 1 - *MOLLUGO OPPOSITIFOLIA* – HABIT (a) WHOLE PLANT (b) PLANT ENLARGED

ANATOMY – ROOT

Transverse section of the root is circular in outline. The outermost layer is single epidermis and inner to it shows a narrow cork made up of 3-4 layers of tangentially elongated and radially arranged suberized cells are present. Next a narrow thin walled parenchymatous cortex is seen. Inner to cortex crescent shaped parenchymatous phloem, followed by 1 to 2 continuous or discontinuous rings of lignified radially arranged xylem consisting of wide, thick walled vessels, fibres and uni to bi-seriate thin walled xylem rays are present. In the center portion parenchymatous pith is seen (Fig. 2 e, f).

STEM

Transverse section of the stem is wavy in outline. It shows a layer of tangentially elongated, thick walled epidermis with thick cuticle bearing glandular trichomes. The trichomes are multi-cellular stalk and uni to bicellular head. The cortex is parenchymatous. The stele constituted of a layer of endodermis, sclerenchymatous pericycle, vascular bundle arranged in the form of a ring around parenchymatous pith. The xylem vessels are single and circular in shape (Fig. 2 c, d,).

LEAF

Transverse section passing through the midrib is flat on the upper and curved at the lower side and shows a centrally located collateral meristele. The section shows the presence of upper and lower epidermis. The palisade tissue occupy the major area of the mesophyll and next the spongy tissue is present. The midrib has vascular bundle with phloem facing upper epidermis and xylem next to it. Rosettes and prisms of calcium oxalate are scattered in the lamina (Fig. 2 a, b).

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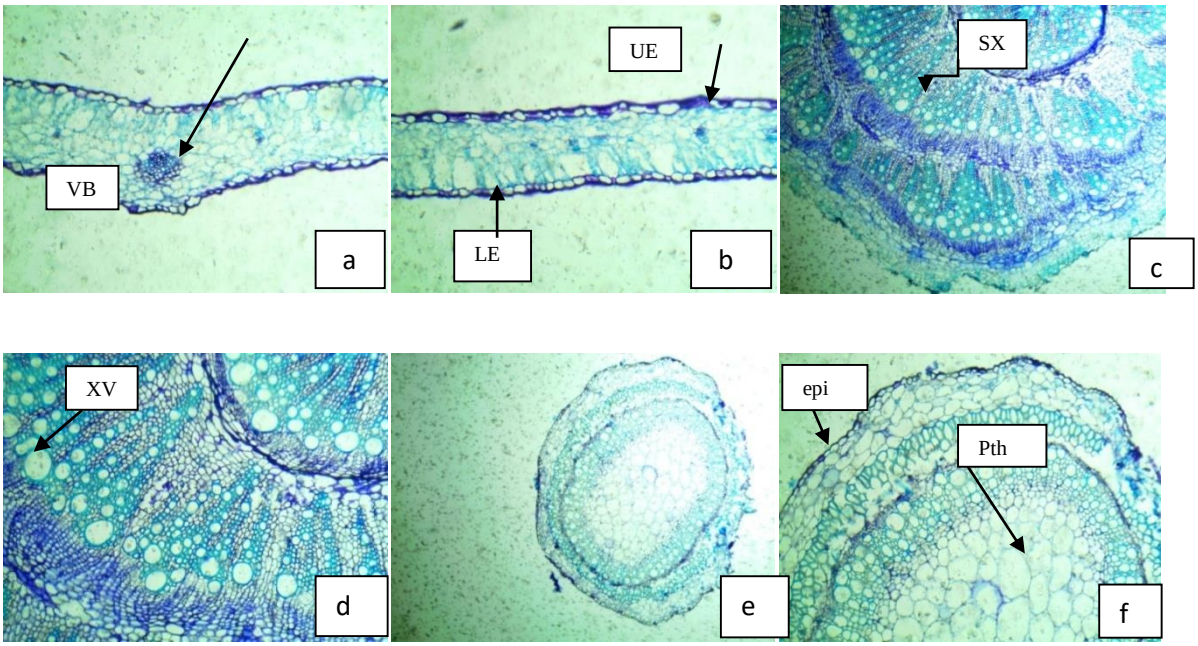

FIGURE 2- ANATOMY OF LEAF, ROOT AND STEM

(a) T.S.OF LEAF THROUGH MIDRIB 10X ; (b)T.S OF LAMINA 10 X; (c) T.S. OF STEM 4X;

(d) T.S. OF STEM 10 X ; (e)T.S OF ROOT 4 X- ENTIRE VIEW;(f) T.S. OF ROOT 10 X

(VB- VASCULAR BUNDLE, UE- UPPER EPIDERMIS, LE- LOWER EPIDERMIS, SX- SECONDARY XYLEM, XV- XYLEM VESSELS, EPI- EPIDERMIS, PTH- PITH)

The above result shows that the morphological characters and anatomical characters serve as an important parameter to identify the specific plant material. The organoleptic characters like leaves are arranged in whorl and spathulate leaf shape and corolla is absent helps in identifying the original plant from adulterants. The macroscopic characters where the outline of root is circular with a narrow cork made up of 3-4 layers of tangentially elongated and radially arranged suberized cells, fibres and uni to bi-seriate thin walled xylem rays are excellent characters by which the plant cane be identified easily. In addition the transverse section of the stem is wavy in outline, glandular trichomes with multi-cellular stalk and uni to bicellular head. The leaf has rosettes and prisms of calcium oxalate scattered throughout lamina (Mammen *et al.*, 2011). All the above mentioned microscopic features are guidelines for botanical diagnosis of the fragmentary samples of *Mollugo oppositifolia* which can be used in Pharmaceutical companies for crude evaluation to find the original crude drug from adulterants (Rabeand Staden, 1997; Dinesh Kumar,2007)

INVITRO STUDIES

A preliminary *in vitro* studies was carried out for *M. oppositifolia* using axillary bud, and stem as explants. Initiation of shoots in nodal explants occurred within 5-8 days of inoculation while it took more than 10 days in basal medium. BAP (2 mg/l) produced highest number of multiple shoots with

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86% of response after 8 weeks of inoculation. The induction of multiple shoot decreased gradually in all other hormones tested. Callus induction began by curling of leaves and callus appeared at the cut ends of the leaf explants within 8-10 days of inoculation. Highest frequency of callus was obtained from the leaf explants inoculated in NAA 3mg/l.

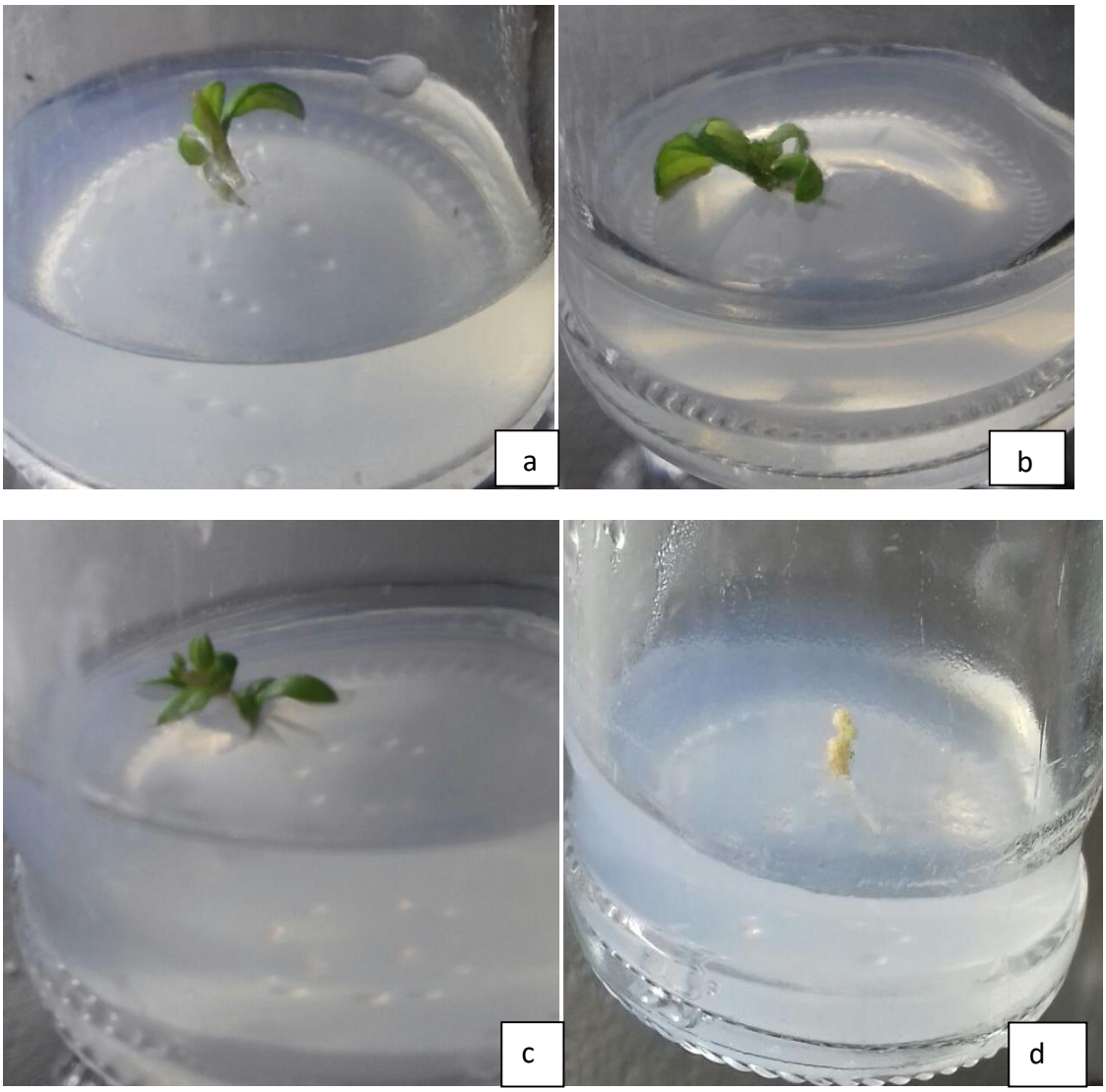

FIGURE 3- *INVITRO* MULTIPLICATION - (a) SHOOT TIP EXPLANT AFTER 7 DAYS OF CULTURE INOCULATED ON MS MEDIUM CONTAINING BAP 2MG/L; b) SHOOT TIP AFTER 2 WEEKS OF CULTURE.; (c) MULTIPLE SHOOTS AFTER 4 WEEKS OF CULTURE; (d)CALLUS FORMATION FROM STEM EXPLANTS AFTER 3 WEEKS OF CULTURE IN NAA 3 MG/L.

The described surface sterilization methods of nodal and stem explants were able to produce 80 -85% aseptic explants. BAP 2.0 mg/ was found to be the best plant growth regulator for multiple shoot induction (Table 1). At lower and higher concentration of BAP a decrease in % response was observed. Comparable results have been showed in many other medicinal plants like *Mollugo nudicaulis*, (Nagesh and Shanthamma,2011), *Caralluma diffusa*, (Kalimuthu *et al* 2014). This study clearly shows that, BAP alone is effective for rapid shoot multiplication.

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TABLE 1 - EFFECT OF PLANT GROWTH REGULATORS ON SHOOT INDUCTION FROM NODAL EXPLANTS OF *M. OPPOSTIFOLIA* AFTER 8 WEEKS OF CULTURE

Hormone Concentration(mg/l)	Explants Responded (%)
BAP	
1.00	72%
2.00	**86%**
3.00	64%
BAP +NAA	
1.00+0.50	40%
2.00 +0.20	40%
2.00 +0.50	52%

TABLE 2 - EFFECT OF PLANT GROWTH REGULATORS ON CALLUS INDUCTION FROM LEAF EXPLANTS OF *M. OPPOSTIFOLIA* AFTER 4 WEEKS OF CULTURE

Hormone	Explants inducing callus (%)
NAA	
Control	20%
1.00	58%
2.00	55%
3.00	**78%**
4.00	56%

CONCLUSION

Morphological and Microscopic studies are useful to establish the botanical identity for the valuable herbal drugs, which forms the basis for the identification and determination of adulterants. In the present study *Mollugo oppositifolia* is used as adulterant for the medicinal plant *Hedyotis corymbosa*. The organoleptic and microscopic characters serve as an important tool to identify the original and the adulterants. The circular outline of root, a narrow cork made up of 3-4 layers of tangentially elongated and radially arranged suberized cells, fibres and uni to bi-seriate thin walled xylem rays provide that the sample is Root of *Mollugo oppositifolia.* The stem shows wavy outline, glandular trichomes with multi-cellular stalk and uni to bicellular head and the leaf has rosettes and prisms of calcium oxalate scattered throughout lamina. All the above mentioned microscopic features

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are guidelines for botanical diagnosis of the fragmentary samples *Mollugo oppositifolia*. These characters help in identifying the adulterants from the original raw drugs supplied to Pharmaceutical companies which is the need of the hour.

The present study reports a rapid propagation of shoots and callus induction. The study on callus induction and direct shoot formation from nodal explants offers the possibility to select different clones for further investigation on physiological, chemical and/or pharmaceutical aspects. During the present investigations protocol for *in vitro* regeneration of *Mollugo oppositifolia* could be considered for large scale multiplication and propagation of this important medicinal plant.

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