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Comparative Pharmacognostic Evaluation of *Munronia Pinnata* (Wall.) Theob. (Meliaceae) and Its Substitute *Andrographis paniculata* (Burm.f.) Wall. Ex Nees (Acanthaceae)

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Abstract Background: *Munronia pinnata* (Wall) Theob. (Meliaceae), a rare, therapeutically important medicinal plant, which is often adulterated by materials of *Andrographis paniculata* (Burm.f.) Wall. ex Nees (Acanthaceae). However, adulteration of *M. pinnata* with *A. paniculata* without scientifically proven data on important quality standards might adversely affect the therapeutic properties of herbal drugs. Methodology: Therefore, the present study was undertaken to establish a comparative quality standards on morphological, anatomical, powder microscopical, phytochemical, physicochemical and antioxidant activity of *M. pinnata* and *A. paniculata* by using established protocols. Principal Findings: Results demonstrated that *M. pinnata* could be distinguished from *A. paniculata* by comparing polymorphic morphological characters, anatomical and powder microscopic characters. Major phytochemical groups were present in leaves, stem and roots of both plants. Results of TLC exhibited the highest number of common spots in leaf (R_f 0.10, 0.23, 0.30, 0.56, 0.86 and 0.96) followed by stem and root extracts for both *M. pinnata* and *A. paniculata*. Both plant species possess notable total antioxidant capacity (TAC) of all three parts tested. However, higher TAC was exhibited in *A. paniculata* compared to *M. pinnata*. Order of increase of TAC was leaf > stem > root for *M. pinnata* and stem > leaf > root for *A. paniculata*. Conclusions/Significance: The presence of certain similarities in major phytochemical groups, and in antioxidant capacity of *M. pinnata* and *A. paniculata* to some extent justifies the use of *A. paniculata* as a substitute for *M. pinnata* in traditional systems of medicine in Sri Lanka which needs to be confirmed after further clinical *trials.*

Keywords: *Meliaceae, acanthaceae, Munronia pinnata, Andrographis paniculata, substitute, phytochemical parameters, antioxidant capacit*

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1. Introduction

Herbal medicine is gradually getting popular among people in both developing and developed countries due to its less /no adverse side effects. Quality evaluation of herbal materials for safety and efficacy by using multi-component systems and acceptable analytical methodologies is fundamental as it strengthens their quality, safety and efficacy [1,2]. With respect to quality control, correct identification of the species concerned from commonly available adulterants or substitutes, in fresh, dried or powdered state is of prime importance [3]. Since adulterants/ substitutes closely resemble the genuine material, macroscopic or microscopic evidences alone cannot always provide evidence for complete identification [4]. On the other hand misidentification of species and the subsequent substitution with unsuitable material could lead to a real danger in the preparation and administration of herbal medicine [5]. Serious adverse affects of substitution and misidentification of species. which are used for the Chinese Traditional Medicine have been reported [6,7]. Therefore, implementation of rigorous standardization procedure for correct botanical identity, part of the plant, appropriate state of maturity, harvesting, processing and storage practices by using multi component analytical techniques is of primary importance in order to avoid harmful adulteration, substitution, contamination and degradation [8].

Munronia pinnata is an expensive, valuable, rear medicinal plant possessing many health claims and being used for the treatment of malaria, recurrent fever, dysentery and purification of blood in traditional systems of medicine in Sri Lanka [9,10]. *Andrographis paniculata*

is commonly available, less expensive medicinal plant used for the treatment of scabies, boils, skin eruptions, and chronic undetermined fevers [11]. Moreover, incorporation of cheaper, abundant substituent /adulterant *Andrographis paniculata* instead of authentic *M. pinnata* is becoming a very common practice in the open herbal markets in Sri Lanka (Personnel communication). Even though, this substitution has been practiced since long times, available information on comparative pharmacognostic, physicochemical, phytochemical and antioxidant capacity of this pair of plants are scares. In the present study attempts have been made to compare gross macroscopical and microscopical characters, physicochemical, physicochemical properties and antioxidant capacity of *M. pinnata* and *A. paniculata* as important pharmacognostic parameters in herbal drug standardization process.

2. Materials and Methods

2.1. Plant Material

Both *M. pinnata* and *A. paniculata* plants were collected from the institutional research plots where authenticated plants were maintained under similar soil and climatic conditions. Herbarium specimens of both plants were prepared and deposited (HTSMP 17 & HTSAP-18) in the institutional herbarium.

2.2. Preparation of Free Hand Sections

Free hand transverse sections were made using razor blades. Suitable sections were selected and taken through an alcohol series (as 30% and 50% alcohol for 5 minutes each) and subsequently strained with 1% safranin in 50% ethanol. Stained material was made into temporary mounts using glycerin. Photomicrographs were taken using a digital camera attached to Olympus, Model CX 31 microscope.

2.3. Powder Microscopy

Powder microscopical studies were carried out following standard protocol with slight modifications [12]. Small amount of ground, whole plant material of both *M. pinnata* and *A. paniculata* were separately mounted on labeled glass slide containing one to two drops of water and glycerol/ ethanol by using moist tip of a needle. After placing the cover slip, it was warmed gently to remove air bubbles and observed under the microscope. Illustrations were made and photomicrographs were taken by using a digital camera attached to Olympus, Model CX 31 microscope.

2.4. Phytochemical Studies

2.4.1. Preparation of Extracts

About 10 g of coarsely powdered plant material of each species was separately extracted in 50mL of methanol by using Soxhlet apparatus. The extract was concentrated at 45°C using rotovapour (Buchi Rotavapour, Type-R-114A29 B-480, Switzerland).

2.4.2. Phytochemical Screening

The phytochemical screening tests for alkaloids, flavonoids. saponins, steroid glycosides and tannins were performed according to the method described by Farnsworth [13].

2.5. Thin Layer Chromatography

The Thin Layer Chromatography (TLC) was performed according to the method described by Stahl [14] with some modifications. About 8 µL of the extract was spotted on TLC plates (Pre-coated silica gel 60 A 20 X 20cm; 0.2mm thickness) and developed using chloroform: dichloromethane: cyclohexane: methanol (5:4:1:0.4) as the mobile phase. They were observed under UV 366 nm and after spraying with Vanillin-Sulfuric acid. Spots were marked, specific colour of the spots was recorded and R_f value for each spot was calculated.

2.6. Determination of Total Antioxidant Capacity

Total antioxidant capacity was determined using Ferric Reducing Antioxidant Power (FRAP) assay according to standard protocol [15]. Methanolic extract (100 µL) was mixed with 900 µL of freshly prepared FRAP reagent of pH 3.6 containing 2.5 mL of 10 mmol/L, 2,4,6-Tripyridyl-s-Triazine (TPTZ) solution in 40 mmol/L, HCl plus 2.5 mL of 20 mmol/L $FeCl_3$ and 25 mL of 300 mol/L acetate buffer. After incubating for 4 minutes, absorbance of the reaction mixture was measured at 593 nm using the spectrophotometer (Shimadzu, UV Mini 1240, Japan). Trolox was used as e standard solution.

2.7. Statistical Analysis

Results of physico-chemical parameters and antioxidant activity were analyzed by general linear model (GLM) ANOVA test followed by Duncan's Multiple Range Test (DMRT) and presented as means ± SE.

3. Results and Discussion

Present study compared the prominent morphological, anatomical, powder microscopical, preliminary phytochemical, physico-chemical parameters and antioxidant capacity of *M. pinnata* and *A. paniculata* which could be used in herbal drug standardization process.

3.1. Morphological Variations

Figure 1. Comparison of morphological features of *Munronia pinnata* (Left) and *Andrographis panicuala* (Right) [1. Mature plant of *M. pinnata*, 2. Mature plant of *A. paniculata*, 3. Different stages of flowering and fruiting of *M. pinnata*, 4. Different stages of flowering and fruiting of *A .paniculata*; A. Flower, B. immature fruit, C. Mature fruit, D. Seeds].

Basic morphological features of *M. pinnata* and *A. paniculata* are shown in Figure 1 and distinguished polymorphic vegetative and reproductive characters are given in Table 1.

As shown in Table 1, flower type, number of petals, number of sepals, flower size, fruit type could be considered as distinguish reproductive characters while leaf, stem and petiole characters exhibit prominent polymorphic vegetative characters. These characters could be used in differentiating *Munronia pinnata* from its adulterant in the raw material.

Table 1. Distinguished polymorphic vegetative and reproductive characters of *Munronia pinnata* and *Andrographis paniculata*

Character	Plant species	
1.Vegetative characters	*Munronia pinnata*	*Andrographis paniculata*
Plant size	Comparatively small (30-40cm)	Comparatively large (up to 100 cm
Habit	Unbranched perennial	Much branched herbaceous annual
Phyllotaxy	Whorled	Opposite
Leaf form	Imperipinnate	Simple
Leaf margin	Entire to dentate	Entire to lanceolate
Leaf apex	Obtuse	Acute
Stem shape	Round	Quadrangular
2.Reproductive characters		
Flower size	Medium	Small
Flower colour	White	White to purple colour
Ovary	Superior	Superior ovary, 2-celled
Fruit type	Capsule	Linear Capsule
Seed colour	Brown	Yellowish brown
Number of seeds per fruit	5-7	Many

3.2. Anatomical and Powder Microscopic Features of *MunroniaPpinnata* and *Andrographis Paniculata*

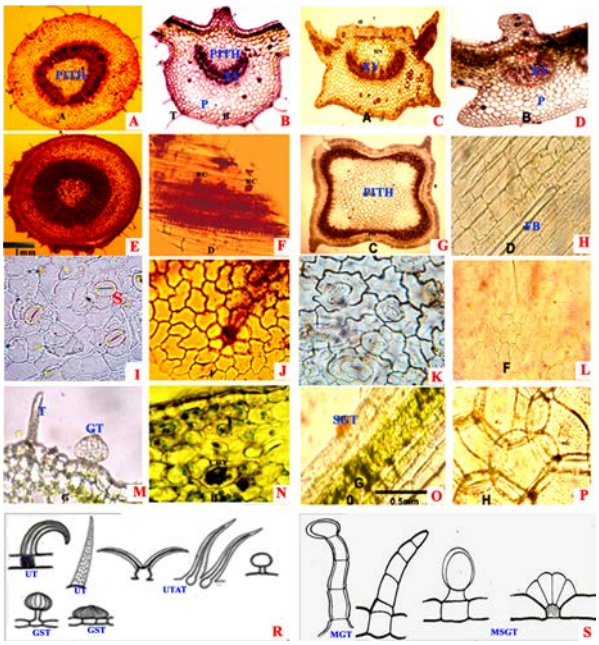

Figure 2. Distinguished anatomical and powder microscopical features of *Munronia pinnata* and *Andrographis paniculata* (Epidermal peels and sections of the leaf midrib, petiole and stem of *M. pinnata* and *A. paniculata* [A-Transverse section of leaf petiole of *M. pinnata*, B-Transverse section of leaf midrib of *M. pinnata*, C- Transverse section of leaf petiole of *A. paniculata*, D- Transverse section of leaf midrib of *A. paniculata*, E- Transverse section of stem of *M. pinnata* F- leaf sections showing different types of crystals; G - Transverse section of stem of *A. paniculata* H- stem longitudinal section of *A. paniculata* showing crystals and pitted fibers I- Lower epidermis with stomata of *M. pinnata*, J - Upper epidermis of *M. pinnata*, K- Lower epidermis with stomata of *A. paniculata*, L- Upper epidermis of *A. paniculata*, M-Leaf midrib section of M. pinnata showing glandular and non glandular trichomes, N-Rosette crystals in phloem tissue, O- Glandular sessile trichome of *M. pinnata*, P- Leaf sections showing different types ofcrystals; R - different types of trichomes present in *M. pinnata*, S - Different types of trichome present in*A. paniculata* T-Trichomes; UE- upper epidermis; LE- lower epidermis; GT-Glandular trichomes; SGT-Sessile glandular trichome; CRY- Rosette crystals; PH-Phloem; P-Parenchyma cells; PL- Palisade; XYXylum;UE-Upper epidermis; UT- unicellular trichome, LE-Lower epidermis; TWF- Thick wall fiber,MGT- multicellular glandular trichome, MSGT- multicellular sessile glandular trichome, UTAT-unicellular two armed trichome].

Important anatomical features of *M. pinnata* and *A. paniculata* are presented in Figure 2.

Table 2 and Figure 2 summarize the distinguished leaf stem and powder microscopic features which are useful for proper identification of *M. pinnata* and *A. paniculata*. Type of stomata, epidermal cell margins, present of different kinds of trichomes, shape of palisade layers, shape of the stem, collenchyma densely at the corners of the stem and availability of different types of crystals were key polymorphic features of leaf of *M. pinnata* and *A. paniculata*.

Table 2. Distinguished polymorphic anatomical and powder microscopic characters of *Munronia pinnata* and *Andrographis paniculata*

Character	Plant species	
Leaf characters	*Munronia pinnata*	*Andrographis paniculata*
Stomatal type	Anomocytic stomata only in lower surface	Diacytic stomata only in lower surface
Epidermal cell	Entire, irregular or hexagonal	Wavy, irregular
Cystoliths	Small in lower epidermis	Large cystoliths in upper and lower epidermis
Trichomes	Simple unicellular, two armed and different types of multicellular stalked and sessile glandular trichomes	Multicellular uniseriate and multicellular glandular stalked and sessile trichomes
Collenchyma cells	Collenchyma in midrib both upper and lower sides	Collenchyma in midrib beneath epidermis
Palisade	Single layer	Columnar type layer
Stem characters		
Shape of the stem	Round	Quadrangular
Vascular bundle nature	Well developed round/ triangular complete thick vascular bundle	Thin vascular layers at corners of the stem
Collenchymas	Many layers	Dense collenchymas stands at angles
Parenchyma cells	Many layers	Large spongy parenchyma cells in the pith
Types of crystals present	Rosette crystals	Needle like crystals
Pith	Small	Large

Presence of leaf fragments with lower epidermis showing anomocytic stomata are prominent character in *M. pinnata* while leaf fragments with diacytic stomata, collumner palisade are characteristic in *A. paniculata*. Presence of solitary as well as clustered crystals, two armed hairs, fragments of single palisade layer, idoblasts attached to parenchyma tissue fragments and oil globules were observed in powder of *M. pinnata*.

Presence of closed or arch shaped vascular rings in leaves and stems, different types of trichomes, anomocytic stomata in lower epidermis have been identified as common features of family Meliaceae. Further, stone cells in cortex as well as pericycle of the cork, various types of solitary or clustered crystals have been observed in the tissues of all organs [16]. They further highlighted the availability of secretary cells, distribution of solitary and clustered crystals as special diagnostic feature of family Meliaceae. Similarly, presence of quadrangular stem with dense collenchyma strands at angles, diacytic stomata and cystoliths in both upper and lower epidermis have been reported as distinguished diagnostic features of *A. paniculata* [17]. Moreover, morphological and anatomical features of leaves have been successfully used for the identification of leaves of *Olivae folium* and its counterparts [18].

3.3. Phytochemical Variations

Thin Layer chromatography (TLC) is the widely used analytical method in herbal drug standardization process due to its simplicity, rapidness and cost effectiveness [19]. In the present study, TLC fingerprints of leaf stem and root extracts of *M. pinnata* and *A. paniculata* were compared. The highest number of common spots both under UV 366 nm (R_f 0.35, 0.50, 0.71, 0.85) and after spraying with vanillin sulfuric (R_f 0.50, 0.78, and 0.88) in both *M. pinnata* and *A. paniculata* was observed in leaves followed by stem and root extracts. Moreover, two spots (R_f 0.16 and 0.70) were common for leaf, stem and root extracts of both plants after spraying with vanillin sulfuric. TLC fingerprints visualized under UV 366 nm exhibited some species specific spots for leaf (R_f 0.56, 0.59), stem (0.57) extracts of *M. pinnata*. Similarly, spots with R_f 0.32 and 0.72 were distinguished for leaf extracts of *A. paniculata*. The presence of similar spots in all three extracts as well as individual extracts indirectly validates the traditional claim of using *A. paniculata* as a substitute for *M. pinnata* since ancient times in traditional systems of medicine in Sri Lanka. In contrast presence of spots with different R_f values may be due to species specific chemical compounds present in different parts of these two species.

As presented in Table 2, leaf, stem and root extracts of *M. pinnata* and *A. paniculata* positively reacted on saponin, alkaloids, tannins, flavonoids and steroid glycosides. Present results are in agreement with previous studies [20] which reported the presence of saponin, alkaloids, tannins, flavonoids and steroid glycosides in *A. paniculata*.

3.4. Physicochemical Parameters

As shown in Table 3, significantly higher values for all tested physicochemical parameters were observed for leaf extracts. In this study, it reveals that Total ash, Water soluble ash, Acid insoluble ash and moisture contents vary in the order as leaves > stem > root in both plants. In the mean while all these are higher in *A. paniculata* rather than *M. pinnata* except water soluble ash. Comparatively higher extractable matter content was exhibited in hot extraction over the cold extraction. The order of extractable matter content varied as leaf>stem> roots in both hot and cold extraction methods. This may be due to enhancement of extraction in hot extraction procedure. Observed higher extractable matter content of leaf are in agreement with previous studies [21], which reported the presence of comparatively higher extractable matter content in leaf extracts of *A. pannuculata*.

Table 3. Phytochemical analysis of different parts of *Munronia pinnata* and its' adulterant *Andrographis paniculata*

Phytochemical	Plant species					
	Munronia pinnata			*Andrographis paniculata*		
	Leaves	Root	Stem	Leaves	Root	Stem
1.Saponin	+	+	+	+	+	+
2.Alkaloids	+	+	+	+	+	+
3.Tanninns	+	+	+	+	+	+
4.Flavanoids	+	+	+	+	+	+
5.Steroid Glycosides	+	+	+	+	+	+

+= Presence; - = Absence

3.5. Antooxidant Capacity

The antioxidant capacity (TAC) of plants is mainly contributed by the varying amount of active molecules/ ingredients present in different parts of the plant. Results of (TAC) showed the presence of antioxidant capacity to a considerable extent in all three plant parts tested. However, higher TAC was exhibited in *A. paniculata* compared to *M. pinnata*.

Table 4. Physico-chemical analysis of leaves, stems and roots of *Munronia pinnata* and its' adulterant *Andrographis paniculata*

Parameter	Plant species					
	Munronia pinnata			Andrographis paniculata		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Total extractable matter (%)	11.99±0.25	1.76±0.04	1.15±0.14	15.62±0.12	8.37±0.21	2.68±0.17
Total ash content (%)	11.94±0.35	4.79±0.16	3.34±0.25	16.31±0.55	6.22±0.0.70	5.65±0.0.21
Water soluble ash content (%)	5.26±0.29	1.58±0.05	1.03±0.25	4.35±1.84	3.55±0.12	2.84±0.13
Acid insoluble ash content (%)	0.85±0.34	0.35±0.04	0.24±0.01	1.81±0.21	0.36±0.10	0.34±0.02
Moisture content (%)	8.92±0.26	9.53±0.20	9.42±0.13	12.10±0.10	11.54±0.19	11.16±0.23

Values are the mean of 4 replicate results are presented as Mean±SE

Order of increase of TAC was leaf > stem > root for *M. pinnata* and stem > leaf > root for *A. paniculata* (Table 4). Presence of prominent antioxidant activity in leaf and stem extracts for *A. paniculata* has been reported by previous workers [21,22]. In the present study pharmacognostical evaluation of distinguished morphological, anatomical, powder microscopical characters, physicochemical and phytochemical properties and *in vitro* antioxidant capacity have been investigated in order to differentiate authentic *M. pinnata* from *A. paniculata.* Procedures of morphological, anatomical powder microscopical, Thin Layer chromatography and antioxidant capacity were adopted from the standard methods described in literature [12,14,15,16,23]. In conclusion, it is clear that *M. pinnata* could be differentiated from *A. paniculata* by comparing above characters.

Table 5. Total Antioxidant Capasity of *Munronia pinnata* and *Andrographis panniculata,* TE- trolox equivalent

Plant part	Antioxidant activity (mg/TE/g)	
	Munronia pinnata	*Andrographis paniculata*
Leaf	13.08±0.41[a]	73.96±0.86[d]
stem	8.67±0.30[b]	99.14±1.51[e]
Root	7.08±0.29[c]	61.33±0.60[f]

Values of different letters are significantly different at 5% significance level

4. Conclusion

Moreover, presence of certain phytochemical groups, and antioxidant capacity of *M. pinnata* and *A. paniculata* justifies the use of *A. paniculata* as a substitute for *M. pinnata* in traditional systems of medicine in Sri Lanka, which needs to be confirmed after further clinical trials. Information gathered through the present study could be directly used for the upgrading of Sri Lankan pharmacopeia.

Statement of Competing Interests

the authors have no competing interests.

References

[1] Ping Li, Lian-Wen Qi., E-Hu Liu., Jian-Liang Zhou., Xiao-Dong Wen., Analysis of Chinese herbal medicines with holistic approaches and integrated evaluation models. *Trends in Analytical Chemistry 27*, 66-77. 2008.

[2] Naumovski, V.R., Wannit Tongkao-on., Benjamin Kimble., Vincent L, Qiao, Lin Beilun., Kong, M. Li., Basil Roufogalis., Yang Depo, Yao Meicun., George Q, Multiple Chromatographic and Chemometric Methods for Quality Standardization of Chinese Herbal Medicines. *World Science and Technology* 12, 99-106 2010.

[3] Springfield, E.P, Eagles, P.K.F, Scott, G, Quality assessment of South African Herbal Medicines by means of HPLC fingerprinting. *J. of Ethnobotany* 101, 75-83. 2005.

[4] WHO, *Quality Control Methods For Herbal Materials,* World Health Organization, 1998.

[5] Opara I, Elizabeth, The efficacy and safety of Chinese Herbal Medicines. *Br. J. Nut*. 91,171-193, 2004.

[6] Chan, T.Y, Critchley, J.A., Usage and adverse effect of Chinese herbal medicines. *Hum. Exp. Toxicol*. 15**,** 5-12., 1996.

[7] Li, X.M, Zhang, T.F, Huang, C.K, Srivastava, K., Teper, A. A., Zhang, L, Schofield, B. H, Sampson, H.A, Food Allergy Herbal Formula-1 (FAHF-1) blocks peanut-induced anaphylaxis in amurine model. *J. Allergy. Clin. Immunol.* 108, 639-646. 2001.

[8] Dennis V.C. Awang, *Standardization of Herbal Medicinal Products.* Proc. XXVI IHC – Future for Medicinal and Aromatic Plants. Eds. L.E. Craker et al. Acta Hort. 629, ISHS. 2004.

[9] Jayaweera, B.M.A, *Medicinal plants (Indigenous and Exotic) used in Ceylon*- part (IV). 59 Natn. Sci. Coun. Sri Lanka. 1982.

[10] Mabberley, *Meliaceae.* In: Dassanayake MD, Forsberg FR. (ed.) A Revised Handbook to the Flora of Ceylon: 229-239 Amerind. Publ. Co. Ltd, New Delhi, India. 1995.

[11] Shahid Akbar, *Andrographis paniculata*: A Review of Pharmacological Activities and Clinical Effects. *Alternative Medicine Review* 16, 66-68, 2011.

[12] Trease and Evans, W.C, Trease and Evans' Pharmacognosy (fifteenth editions ed.). Cambridge university press, Great Britain. 2006.

[13] Farnsworth, N.R, Biological and phytochemical screening of plants. *Journal of Pharmaceutical Science*. 55, 225-276, 1966.

[14] Stahl, E, Thin Layer Chromatography. A Laboratory hand book. 2nd Ed. 854-859 Springer international student edition, 1969.

[15] Benzie, I.F. and Strain, J.J, The Ferric Reducing Ability of Plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal. Biochem*., 239, 70-76 1996.

[16] Metcalfe, C.R., Chalk, L, *Anatomy of Dicotyledons* (First edition ed., Vol. 1) London : Oxford university Press. 1957.

[17] Sudhakaran, 2012. Botanical pharmacognosy of Andrographis paniculata (Burm.F. Wall.Ex. Nees. *Pharmacognosy Journal* 4 , 1-10,2012.

[18] Branislava Lakušić, Violeta Popov, and Dušanka Runjajić-Antić, Morpho-anatomical characteristics of the raw material of the herbal Drug *olivae folium* and its counterfeits. *Arch. Biol. Sci*., Belgrade, 59 (3), 187-192 2007.

[19] Pravin H. Nikam, Joseph Kareparamban., Aruna Jadhav and Vilasrao Kadam., Future Trends in Standardization of Herbal Drugs. *Journal of Applied Pharmaceutical Science,* 2, 38-44, 2012.

[20] Manoj Kumar Pandey, Singh, G.N., Rajeev, K.R., Sharma and Sneh Lata, Physicochemical standardization of *Andrographis paniculata* (nees): an ayurvedic drug Inventi Impact: *Pharm Analysis & Quality Assurance* 2011. [cited 2013 Jun 22]. Available From http://www. inventi.in/Article/paqa/116/11.aspx

[21] Suganya Tachakittirungrod, Siriporn Okonogi, Sombat Chowwanapoonpohn. Study on antioxidant activity of certain plants in Thailand:Mechanism of antioxidant action of guava leaf extract. *Food Chemistry* 103, 381-388, 2007

[22] Eugine Leo Prakash, S. and R. Manavalan, Development, characterisation and/toxicity evaluation of nanoparticles of Andrographolide. *Int J Pharm Sci,* 4, 497-501, 2012.

[23] Mondal, A.K, *Advanced plant taxonomy;* New Delhi: New central Book agency. 2009.