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Research Paper

Anthelmintic prospective of *Barringtonia acutangula (Lacithedaceae)*: An ultra-structural and dose dependent efficacy against avian intestinal tapeworm *Raillietina spp.*

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ABSTRACT: *The present study attempts to investigate the anthelmintic potentiality of a traditional medicinal plant Barringtonia acutangula (L.) Gaertn. (Family: Lacythedaceae) used by the local tribal population of South West Bengal. The methanol bark extract of the selected plant species was studied in vitro, in a dose-dependent efficacy against a group of avian intestinal tapeworm parasite Raillietina spp. (Cyclophyllidea). The effect of the methanol bark extracts on the tegument ultra-structure was observed under scanning electron microscopy. An in-vitro dose-dependent study was performed with different concentrated doses against the model cestode. Praziquantel, a broad-spectrum anthelmintic and Genistein derived from Flemingia vestita (Fabaceae), has been used as a reference drug. A positive control group of tapeworms were acclimatized and maintained at laboratory condition, incubated with 0.9% PBS (Phosphate buffer saline). Time taken for paralysis and death was recorded and compared with reference drugs. Experimentally obtained data were analyzed statistically, to know the significant difference between mean paralysis and death time ($P < 0.05$).*

Key Words: *Barringtonia acutangula, anthelmintic, Praziquantel, ultra-structure, Raillietina spp.*

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I. INTRODUCTION

The foundation of traditional medical practices that effectively control a wide range of human diseases and ailments is plants and their byproducts (Mahmoud and Gairola 2013). Indian oak or *Barringtonia acutangula* (L.) Geartn. (Lacithidaceae), also called "hijal" in Bengali, is a moderately sized annual herb that is found along the banks of rivers, streams, and canals in China, India, Bangladesh, Thailand, and other oriental countries. According to Nath *et al.* (2010) *B. acutangula* (L.) Gaertn. has long been employed in fish rearing. The decoction of the plant is used by local tribal people in West Bengal to treat toothaches and gum problems. According to earlier studies, plants from many different families, including the Lamiaceae, Asteraceae, Malvaceae, Fabaceae, Verbenaceae, Solanaceae, Boraginaceae, and Lauraceae, are used as treatments for gastrointestinal illnesses (De la Cruz-Jiménez *et al.* 2014). The bark of *B. acutangula* contains chemical constituents with antibacterial, anthelmintic, and antioxidant properties (Sultana *et al.* 2019). The leaf extract of *B. acutangula* was also used as reducing agent to synthesize silver nano particles in the bioreduction procedure for green synthesis (Porrawatkul *et al.* 2017). The methanolic fruit peel extract of *Limonia acidissima* (Rutaceae) showed anthelmintic potentiality against *Paramphistomum cervi* (Azad *et al.* 2019). Aqueous extract of leaf and bark of *B. acutangula* was examined for hepatoprotective studies against ethanol induced hepatic stress in rat (Mishra *et al.* 2011; Rashmi and Shenoi 2020). Physicochemical parameters of this selected plant species provide the information of having tannins, saponins, phenols and flavonoid compounds (Vidya and Shingadia 2017). The juice from this plant by-product is used to treat diarrhoea (Pandey 2018). Studies on brine shrimp lethality bioassay reveal that extract of this plant's aerial part has the most toxins (Md. Asaduzzaman *et al.* 2015). Modern management practices that include higher stocking densities of commercial anthelmintics potentially represent a more serious threat than they did before the introduction of broad-spectrum anthelmintics. In these circumstances, naturally derived anthelmintics are much more sustainable and suitable

against tapeworm infections. Therefore the present study aims for the first time to find out the anthelmintic property of *B. acutangula* against a group of tapeworm species belonging to the order cyclophyllidea.

II. MATERIALS AND METHODS

2.1 Plant material and extraction: The stem bark of B. acutangula was collected from tropical regions, during its flowering season. The collected bark portion was washed first with tap water, followed by distilled water, and air dried in shadow for about 5 days. Drying is done to lower the moisture contents of the bark for safe storage and further processing. The dried portion was then ground and placed into a glass jar containing 100% methanol. Following this, after a period of 9-10 days, supernatant was collected and filtered. Consequently, a few days later, semi-liquid sediment was obtained and collected which eventually became a red powder extract. This extract is stored at 4°C for further experimental procedures.

2.2 Experimental organism: Anthelmintic activity was assessed on a group of model cestode Raillietina spp. (Family: Devainideae), collected from the infected foul (Gallus gallus domesticas) intestine. The parasitic specimens were collected from the local abattoirs and washed with physiological saline (PBS, pH 7.2). Isolated cestodes were observed under a light microscope to be assured of having clearly movable scolex and a long chain of proglottids. Helminth experts identified flat-bodied cestodes under consideration of their morphology, habitat and ultrastructure. Isolated tapeworms were kept in an incubation media at 37±1°C containing 0.9% PBS (Phosphate buffered saline) at pH 7.2 (Kar *et al.* 2014).

2.3 Chemicals: All chemicals used for the experiment are purchased from Merk chemical and SRL IndiaPvt.ltd.

2.4 Dose dependent mortality test: Four different concentrated doses (5mg/ml, 10mg/ml, 15mg/ml and 20mg/ml) of previously derived plant extract were prepared and diluted with the required amount of 0.9% PBS and 1% DMSO (Dimethyl sulphoxide) in a temperature controlling magnetic stirrer. Petri dishes containing two healthy cestodes of approximately the same size and weight, having movable scolex were treated with the above-mentioned different dosages. Paralysation was confirmed by observing immobilization of all suckers around the scolex, observing under a light microscope with an eyepiece 10x (20mm field of view) and objective lens 4x (Olympus). A temperature-controlled magnetic stirrer was used to generate and dilute four separate concentrated doses of previously derived plant extract (0.9% PBS, 1% DMSO, and 5mg/ml, 10mg/ml, 15mg/ml, and 20mg/ml). Two healthy cestodes with movable scolex that were on petri dishes and were of roughly equal size and weight were given the various dosages described above. A light microscope with an eyepiece magnification of 10x (20mm field of view) and an objective lens magnification of 4x (Olympus) was used to observe the immobilization of all suckers around the scolex in order to confirm paralysis. By putting the parasites in a slightly warmer PBS solution at around 50°C, death is guaranteed.

Two distinct sets of test methodologies are used to determine efficacy. First, the several parasites of the same size and weight were given the identical doses (5mg/ml, 10mg/ml, 15mg/ml, and 20mg/ml). Second, a specific dose—5mg/ml—is administered to three sets of Petri dishes containing two parasites each to determine the mean paralysis and death time. The parasites were maintained during efficacy testing in conditions that were as close to the intestine of the bird G. domesticus as feasible (37°C temperature incubation and pH 7.4). Finally, treated and untreated parasites were cleaned with PBS, preserved in 10% neutral buffered formalin solution, and maintained at 4°C for future research on changes to surface ultrastructure.

2.5 Scanning Electron Microscopy Study: In order to understand the changes in the surface ultrastructural organization among treated cestodes, specimens were prepared for scanning electron microscopy, following the method described by Ash et al. 2012, with slight modifications. The worms from the two groups—the untreated and treated—were fixed in 10% neutral buffered formalin and maintained at 4 °C for 24 hours. The worms were then washed in PBS, dehydrated with increasing grades of alcohol, and finally dried in pure dried acetone. Following critical point drying (CPD) with liquid carbon dioxide as a transitional fluid, specimens were air dried at ambient temperature (25°C). In a ZEISS EVO-MA 10 model electron microscope, samples were then mounted on a metal stub, coated with platinum (Pt), and studied at various electron high tension values (EHTs) (15.00 kV and 20.00 kV) and magnifications.

III. RESULTS AND DISCUSSION

3.1 Dose-dependent study: All applied doses in an in-vitro efficacy test have an impact on the worms' movement. Every worm exhibits paralysis followed by death in every iteration of the experiment. During efficacy, light microscopically observation suggests scolices and strobila of cestodes move faster immediately after exposure to crude extract. Gradually the scolices of the worm become immotile and the blinking of suckers stopped when paralyzed, but their highly segmented body proper, strobila, shows little but continuous movement. Proglottids of long-segmented strobila show maximum movability during first contact with the methanolic extract. Movability becomes low with the gradual increase of time.

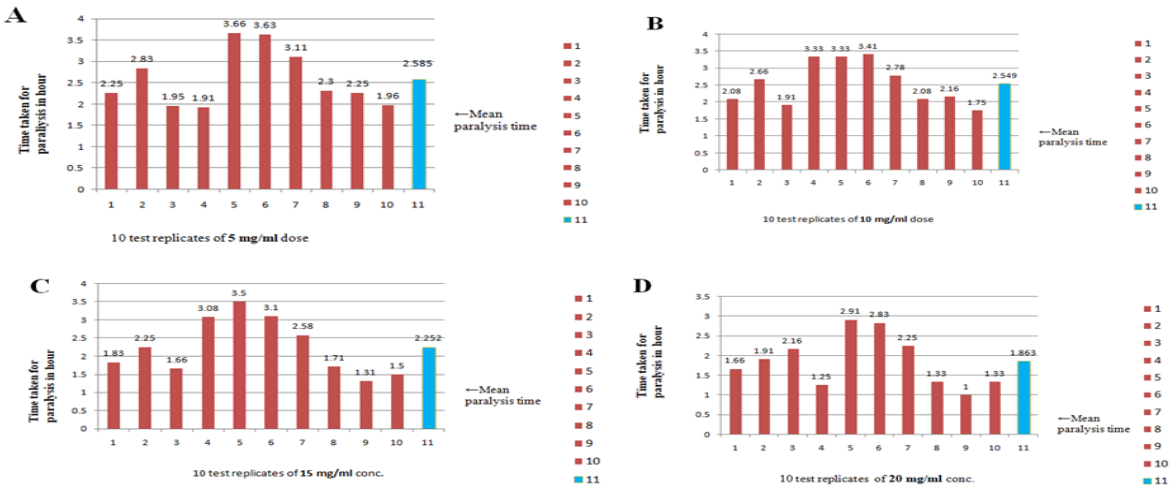

Figure-1: Represents paralysis time of 4 set of different concentrations (A)5mg/ml, (B)10mg/ml, (C)15mg/ml, (D)20mg/ml, each with 10 separate samples.

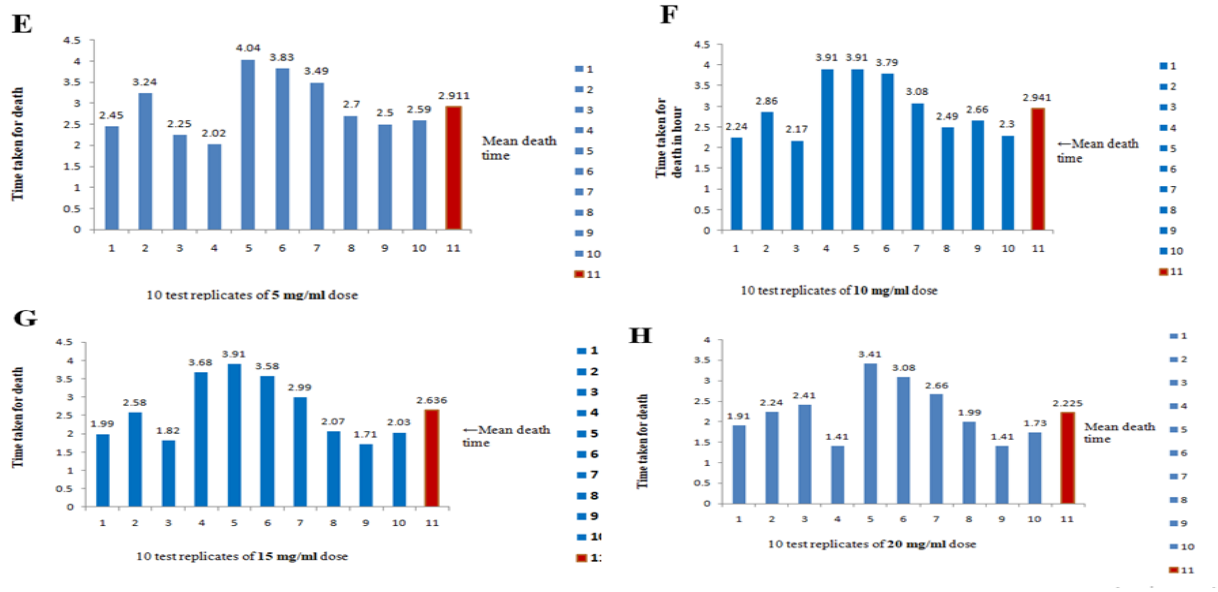

Figure-2: Represents death time of 4 set of different concentrations (E)5mg/ml, (F)10mg/ml, (G)15mg/ml, (H)20mg/ml, each with 10 separate samples.

Four different doses of 5mg/ml,10mg/ml,15mg/ml and 20mg/ml concentration shows mean paralyze times 2.585h,2.549h,2.252h,1.863h (Figure-1: A,B,C,D) and mean death time 2.911h,2.941h,2.656h,2.225h respectively

(Figure-2: E,F,G,H). In Figure-1:D and Figure-2: H shows the maximum significant effect in 20 mg/ml concentration in which the mean paralysation and death time are 1.863h and 2.225h, respectively. According to morphological analysis, the color of the tegument and the shape and size of the parasites change. Controlled parasites in 0.9% PBS on petri plates produce numerous knuckles and nodes and become attached; in contrast, treated cestodes remain free from one another and shrink in size and shape as a result of physiological stress. Statistical analysis of mean death time shows null hypotheses to be rejected, and the difference between the mean death time is considered significant at ($P<0.05$) with df=5. The corresponding death times of the reference drugs praziquantel and genistein for a dose of 0.5 mg/ml are 0.39 h, 0.44 h, 0.44 h (mean 0.42 h) and 1.5 h, 1.58 h, 1.55 h (mean 1.54 h), respectively. The mean death time at the most effective and significant dose of B. acutangula extract (20 mg/ml) was compared with reference medications (Figure 3:A). Tests conducted in vitro point to a dose-dependent mortality, where a lower concentration indicates a long time to live and a larger concentration indicates a short time to live (Figure 3:B).

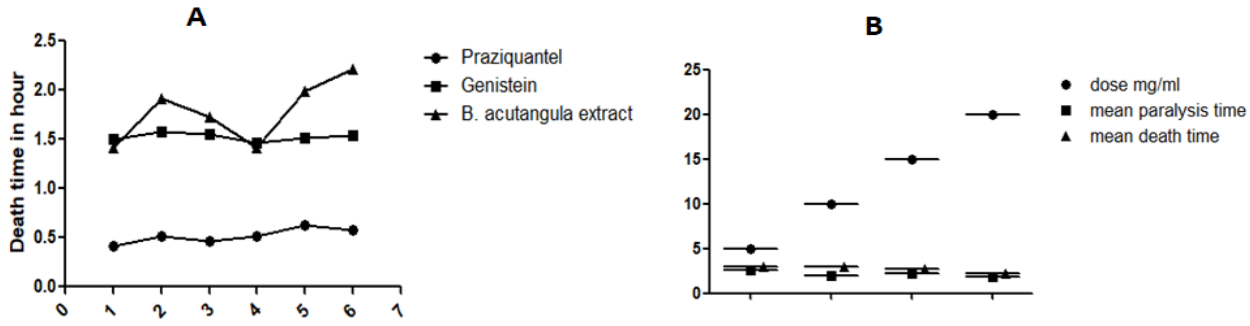

Figure-3: (A) Represents a comparative mortality time of *Raillietina spp.* against similar concentration doses (**1.5mg/ml)** of Praziquantel, Genistein and methanolic extract of *B. acutangula* **(B)** Graphical representation indicating decrease time of paralysis and death with increasing concentration of methanolic *B. acutaungula* bark extract.

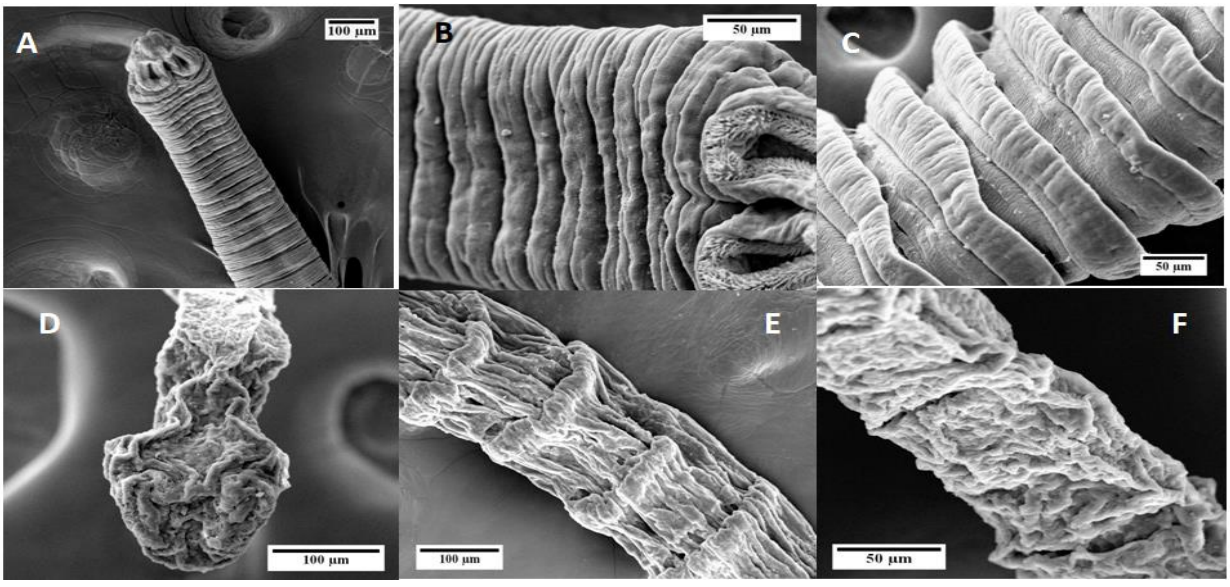

Figure-4: Specified as A, B and C shows scanning electron micrograph of control parasite whereas figure specified D, E and F shows scanning electron micrograph of parasite treated with *B. acutangula.* Presence of undisturbed suckers (A), clear arrangements of microthriches within the suckers (B) and an uninterrupted positioning of individual proglottids segments (C). Destroyed scolex with disorganized suckers blended with neck region of the parasite (D), Disrupted strobila with massive distortion of individual proglottids (E) and (F).

3.2 Ultrastructural alterations: Electron microscopical photograph shows massive disorganization in tegument architectures of treated parasites (fig-4; D,E,F provided scale bar 100 µm, 100 µm and 50 µm respectively**)**

compared to the untreated parasites (fig-4; A,B,C provided scale bar 100 µm, 50 µm and 50 µm respectively**)**. Hooks and microtriches around the scolex were destroyed. Hence they decrease their surface area of attachment and causes disturbance in nutrient absorption. Individual segments of strobila became squeezed, forming several layers of rigid lines one upon another. Some regions of the tegument become ruptured, split and disintegrated. The outer epidermis of the experimental control specimen was soft, and the sucker and rostellum were unaltered. In treated worms the outer epidermis of the parasite becomes rough, and the tegumental surface becomes contorted, which may be due to the presence of saponin, a toxin found in most *B. acutangula* leaves and barks (Rumampuk *et al.* 2003). Control tapeworm shows smooth, fine, organized and orderly arranged rostellum, sucker, microtriches and other ultra structures in the parasite's head and strobila portion. The efficacy test shows the demolition of hooks, suckers and highly segmented body proper, which may be due to functional loss of the central nervous system. Studies on swiss albino mice revealed the methanolic leaf extract of *B. acutangula* possesses significant central nervous system (CNS) depressant activity (Balaji *et al.* 2012). Electron microscopic observation of tapeworms treated with *B. acutangula* extract revealed alteration in tegumental ultra-structure with massive destruction of whole body organization from scolex to individual proglottid segments forming wriggle-like structure.

IV. CONCLUSION

The behavioural approach of fowl intestinal tapeworm is quite similar in both treatment cases with *Barringtonia acutangula* and reference drug Praziquantel. However,the synthetic drug's effect is more efficient compared to the methanolic bark extract. The dose-dependent and ultra-structure efficacy results support the anthelmintic potentiality in this current anthelmintic study. Moreover, from the present results, it can be implicated that the rising concentration of the doses can damage the morphology of the parasite by gradually penetrating from the tissue level to the cellular level of organization, and the central nervous system is becoming non-functional. The result of the present study suggests that *Barringtonia acutangula* (L.) Gaertn. has a great anthelmintic potential and its scientific validation is evident from the current anthelmintic efficacy.

REFERENCES

[1]. Asaduzzaman Md., Rana, S. Md., Hasan S.M. R., Hossain M. Md. and Das N.(2015). Cytotoxic (brine shrimp Lethality bioassay) and antioxidant investigation of *Barringtonia acutangula* (l.).International Journal of Pharma Sciences and Research, **6**(8):1179-1185.

[2]. Ash A, Scholz T, Chambrier A, Brabec J, Oros M, Kar P.K., Chavan S.P. and Mariaux J. (2012). Revision of Gangesia (Cestoda: Proteocephalidea) in the Indomalayan Region: Morphology, Molecules and Surface Ultrastructure. PLOS ONE, **7**(10): e46421.

[3]. Azad A.K., Islam F., Md. Faysal, Islam S., Sugandha N. J., Saha S. and Isah S. I.(2019).Phytochemical Investigation And Comparative Anthelmintic Activity of Between Methanol And Acetone Extract Of *Limonia acidissima* L (Fruit Peel). Pharmacologyonline, **2**:241-247.

[4]. Balaji P., Thirumal1 M., Kumudhaveni B., Kishore1 G. and Aliya1 A (2012). Central nervous system depressant activity of *Barringtonia acutangula* (Linn.) Gaertn. Scholars Research Library; **4**(6):1786-1792.

[5]. De la Cruz-Jiménez L., Guzmán-Lucio M. and Viveros-Valdez E. (2014).Traditional Medicinal Plants Used for the Treatment of Gastrointestinal Diseases in Chiapas, México. World Applied Sciences Journal,**31** (4): 508-515.

[6]. Kar P.K., Murmu S., Saha S., Tandon V., and Acharya K. (2014). Anthelmintic Efficacy of Gold Nanoparticles Derived from a Phytopathogenic Fungus, *Nigrospora Oryzae*. PLOS ONE, **9**(1): e84693.

[7]. Mahmoud T and Gairola, S. (2013).Traditional knowledge and use of medicinal plants in the Eastern Desert of Egypt: a case study from Wadi El-Gemal National Park. Journal of Medicinal Plants Studies, **1**(6): 10-17.

[8]. Mishra S., Sahoo S., Rout K K., Nayak S K., Mishra S K and Panda P K (2011). Hepatoprotective effect of *Barringtonia acutangula* Linn. leaves on carbon tetrachloride-induced acute liver damage in rats. Indian Journal of Natural Products and Resources, **2**(4): 515-519.

[9]. Nath A.J., Rout A. and Bhattacharya P.P. (2010). Traditional use of *Barringtonia acutangula* (L.) Geartn. in fish farming in Chatla floodplain of Cachar, Assam. Indian Journal of Traditional Knowledge, **9**(3):544-546.

[10]. Porrawatkul P., Nuthong W., Pimsen R. and Thongsom M. (2017). Green synthesis of silver nanoparticles using *Barringtonia acutangula* (L.) Gaertn leaf extract as reducing agent and their antibacterial and antioxidant activity. The Journal of Applied Science.**16:**75-81.

[11]. Pandey N.K. (2018). Medicinal Plant Resources of South West Bengal. (3):103.Research wing Director of forest, Govt.of West Bengal,Kolkata. India.

[12]. Rashmi K. and Shenoy K. B. (2020). Hepatoprotective studies of aqueous leaf and root extracts of *Barringtonia acutangula* (L.) Gaertn. against ethanol induced hepatic stress in rats. Indian Journal of Traditional Knowledge. **19** (1): 152-157.

[13]. Rumampuk R. J., Pongoh E.J., Tarigan P., Mander P., A.J. H. L. N (2003). A triterpene ester saponin from the seed of *Barringtonia asiatica*. Indonesian Journal of Chemistry, **3** (3): 149-155.

[14]. Sultana N.A., Aovi F.I. and Rahman S. (2019). Bioactivity assessment of a widely distributed mangrove plant: *Barringtonia acutangula*. Pharmacologyonline, **3**: 301-308.

[15]. Vaidya M. and Shingadia H. (2017). Pharmacognostic study of *Barringtonia acutangula* (linn.) Gaertn. World Journal of Pharmaceutical Research, **6**(7): 1001-1009.