ANTIFUNGAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *DESMODIUM GANGETICUM (*L.) DC.: AN ECONOMIC IMPORTANT PLANT OF NARAYANPUR, BASTAR, CHHATTISGARH

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ABSTRACT

In the present investigation preliminary phytochemical screening and antifungal activity of petroleum ether, chloroform, acetone and aqueous leaf extracts of Desmodium gangeticum (L.) DC. was examined. Antifungal activity against six pathogenic fungal strains viz., Microsporum gypseum (MTCC NO 4524), Trichoderma viride (MTCC NO 793), Aspergillus niger (MTCC NO 281), Curvularia lunata (NCFT), Cladosporium oxysporum (NCFT), Candida albicans was performed by using Poison food technique. Preliminary phytochemical screening revealed the presence of alkaloids, phenols, reducing sugar, saponins, tannins, terpenoids, flavonoids, fixed oils and anthraquinone whereas cardiac glycoside was absent in all four solvent extracts. One-way ANOVA analysis of antifungal activity showed significant difference of ($p \leq 0.05$). Result revealed in acetone leaf extracts of D. gangeticum showed maximum growth inhibition % at 1000µl conc. on A. niger (91.55 ± 0.44) which was more than the value of the standard antibiotics Amphotericine- B (87.55 ± 1.77) followed by acetone leaf extracts showed maximum growth inhibition % at 1000µl conc. on T. viride (87.11 ± 0.44) which was nearest to the value of the standard antibiotics Amphotericine- B (88.89 ± 0.44), chloroform leaf extracts showed maximum growth inhibition % at 1000µl conc. on C. oxysporum (84.25 ± 4.62) which was more than the value of the standard antibiotics Amphotericine- B (82.18 ± 1.51), chloroform leaf extracts showed maximum growth inhibition % at 1000µl conc. on C. lunata (70.7 ± 8.26) which was nearest to the value of the standard antibiotics Amphotericine- B (84.41 ± 0.53), acetone leaf extracts showed maximum growth inhibition % at 1000µl conc. on M. gypseum (68.42 ± 0) which was nearest to the value of the standard antibiotics Amphotericine- B (71.93 ± 1.75), aqueous leaf extracts showed maximum growth inhibition % at 1000µl conc. against C. albicans (62.96 ± 1.85) which was nearest to the value of the standard antibiotics Amphotericine- B (72.22 ± 0). Three-way ANOVA analysis showed no significant difference of ($p \leq 0.01$) against fungal strains. Therefore, D. gangeticum (L.) DC. can be used as a source of new antifungal agent and as a source of new drug as it contain no. of bioactive phytocompounds which have been used as a source of medicine long back with no side effect and an ecofriendly mode of treatment.

Keyword: *Desmodium gangeticum (L.) DC., antifungal activity, growth inhibition percent (%), SPSS analysis.*

1. INTRODUCTION

Desmodium gangeticum Linn. DC. belonging to the family Fabaceae, subfamily- Papillionaceae popularly known as 'Shalaparni' is a perennial shrub. Fabaceae consists of about 20,000 species and about 170 species are widely distributed in tropical and subtropical species growing throughout India. Plant is an erect or ascending under shrub, grows 2 to 4 feet. The stem is angular, woody with numerous branches. Leaves simple, membranous, oblong, obovate or lanceolate, acuminate covered with gray color numerous trichomes. Flowers are small, purple or white in color. Flowers 2 per bract, deep violet or white in axillary and terminal panicles. Raceme 20-50 flowered. Seeds are

small, pale yellow, kidney- shaped. The lateral roots appear yellow with smooth texture. Fruits glabrescent, linear pod, moniliform, reticulate, as long as broad, with hooked hairs, joints separate when ripe into indehiscent one seeded segments, seeds compressed and reniform. Its flowering- fruiting season is during the month of March to December (Mahajon *et al.,* 2015).

Whole plant, roots and bark are used as medicine. The root of the plant, known in trade as 'shaalparnimool' is common ingredient in ayurvedic preperations. It is used as an anti-asthamatic, bronchitis, aphrodisiac, anthelmintic, astringent to the bowels, cures typhoid fevers due to mental disorders, inflammations and piles (Vedpal *et al.,* 2016). It is also use to cure diarrhea, chronic fever, biliousness, cough and vomiting. It is also used in treating pyorrhea. The seeds are powdered and the paste is used in skin eruptions (Pullaiah., 2012). To cure eczema infection leaf paste applied topically. Root decoction in treatment of dysentery, diarrhea. Root paste applied topically: treatment of toothache; leaf- headache. Root chewed to cure premature ejaculation (Kosalge *et al.,* 2009; Tabuti *et al.,* 2003).

Pharmacologically, it has been reported that the plant is found to possess various biological activities such as anti-inflammatory, analgesic and antipyretic activity, anti-ulcer, cytoprotective and anti-secretary activity hypocholesterolemic, anti-diabetic activity, anti-leishmanial activity and anti-implantation activity along with it is effective as anti-writhing and central nervous system (CNS) depressant activity (mishra et, in improving memory and in healing different types of wounds. 5-methoxy-N,N-dimethyltryptamine; N,N-dimethyltryptamine their N-oxides, N-methyl tetrahyroharman from aerial parts and alkaloids active constituents are reported. Roots afforded pterocarpanoids- gangetin, gangetinin, desmodin and several alkaloids. The aerial portion gave indol-3-alkylamines and their derivatives. Because of its great medicinal importance and incessant use in India facing vulnerable status (www.iucn.org) and also in Chhattisgarh state facing status of vulnerable condition (http://envis.frlht.org).

The present investigation focused to evaluate the preliminary phytochemical screening and *in vitro* antifungal activity potential against six pathogenic fungal strains using different solvent extract of leaves of *Desmodium gangeticum* (L.) DC..

2. MATERIALS AND METHODS

Collection of plant sample

Mature green and disease free leaves of *Desmodium gangeticum* (L.) DC. were collected from Herbal Garden, Bakhrupara Kasthagar and tropical dry deciduous forest area of Narayanpur District during their flowering seasons (year: 2015 - 2016). Plants were identified by Vaidraj Ratan Dhar Department of Naturopathy and Yogic Science (Govt. Regd. No - MAH/394/06/THA).

Preparation of crude extract

Leaves were washed 2-3 times with running tap water and rinsed twice with distilled water. Air dried under shade at room temperature for 1-2 weeks till the leaves become brittle enough to break easily. After complete drying plant parts were crushed to fine homogenous powder using mechanical grinder and transferred into air tight poly bags with proper labeling and stored at room temperature. Extraction was carried out by Soxhlet procedure as described by Hozowitz., 1984. Coarse powders of *D. gangeticum* (L.) DC. was subjected to solvent extraction method at a temperature not exceeding their boiling point. Twenty – five grams of powder was uniformly packed into a thimble prepared from Whatman No. 1 filter paper. Extraction was carried out for both aqueous and organic solvent (*viz.,* petroleum ether, chloroform, acetone) of increasing polarity. 250 ml of extracting solvent in flask is heated, and its vapours condense in condenser. The process of extraction was continued untill the solvent in thimble tube of an extractor become colorless. After that the solvent extracts were filtered with the help of Whatman's No.1 filter paper (Yadav and Agrawala., 2011). After filtration, the beaker is kept in waterbath and heated at 40-50°C till all the solvent got evaporated and extract was referred to as the crude extract (Muthukrishnan and Venkatachalan., 2012). Crude extracts was kept in refrigerator at 4°C for further use.

PRELIMINARY PHYTOCHEMICAL TESTS (Harbone, (1973); Trease and Evans (1989); Sofowora, (1993))

1) Test for alkaloids (Mayer's test): 6ml. of extract was mixed with 6ml 1% HCl in stream both then it was filtered. 1ml of Mayer's reagent was added. Presence of turbidity shows presence of alkaloids. Further addition of a few drops of olive oil to form an emulsion confirmed the presence of alkaloids.

2) Test for phenol (Ferric chloride test): 2ml. of the crude extract was added to 4ml distilled water then few drops of 10% ferric chloride were added. Appearance of blue or green color indicated the presence of phenol.

3) Test for carbohydrate - reducing sugar (Fehling's test): 1gm of the extract was dissolved in 10 ml distilled water. This extract was boiled with Fehling solution A and B in test tube color changes were observed. Presence of brick red color indicated the presence of reducing sugar.

4) Test for saponins (Froth test): 0.5gm of the extract was dissolved in 5 ml distilled water. The mixture was shaked vigorously. Formation of stable persistent forth shows the presence of saponins further addition of 6 drops of olive oil while shaking forms an emulsion confirming the presence of saponins.

5) Test for tannins (Ferric chloride test): 0.5gm of the extract was dissolved in 10 ml of distilled water a few drops of 1% ferric chloride solution was added to obtain a brownish green or blueblack precipitate confirms the presence of tannin.

6) Test for terpenoids (Salkowski's test): 0.5gm extract was dissolved in 2ml of chloroform then 3ml concentrated sulfuric acid was added. A reddish brown color in inter phase indicates the presence of terpenoids.

7) Test for flavonoids (Ammonia reduction test): 5ml dilute ammonia was added to 5ml extract then 5ml concentrated sulfuric acid was added formation of yellow color shows the presence of flavonoids.

8) Test for Fixed oil (Filter paper test): A small quantity of the extracts was pressed between 2 two filter papers. Appearance of oil strain on the paper indicates the presence of fixed oils and fat.

9) Test for cardiac glycosides (Keller-Killiani test): 2.5 gm of extract was added to 2.5 ml distilled water 1 ml glacial acetic acid containing a few drops of ferric chloride was added then 0.5 ml of concentrated sulfuric acid was added presence of brown ring at the inter phase indicate the presence of deoxy sugar. A violet ring below the brown ring was observed confirming the presence of cardiac glycosides.

10) Test for anthraquinone (Borntrager's test): 2.5 gm extract was dissolved in 5ml of conc. Sulfuric acid and filtered. The filtrate was dissolved in 2.5ml of chloroform. Chloroform layer was pipetted into a tube and 0.5 ml of 10% diluted ammonia was added formation of pink red or violet color shows the presence of anthraquinone.

Fungal strains

The test organisms used were *Microsporum gypseum* (MTCC NO 4524), *Trichoderma viride* (MTCC NO 793), *Aspergillus niger* (MTCC NO 281), *Curvularia lunata* (NCFT), *Cladosprium oxysporum* (NCFT), *Candida albicans* (Govt. Bhim Rao Ambdkar Hospital Raipur (C. G.)).

Viability of the organisms was maintained by regular transferring a loop full of filamentous fungi into freshly prepared Potato dextrose agar media (PDA) and Sabouraud Dextrose Agar (SDA) medium. Fresh cultures were incubated without agitation for 48 to 72 hours at 26 ± 2°C and stored at 4°C in refrigerator. Test organisms were repeatedly sub- cultured in order to obtain pure isolation (Doherty *et al.,* 2010).

Medium preparation:

Potato (peeled) = 200.0g; Dextrose = 20.0g; Agar – Agar = 15.0g; Distilled water = 1000ml. Peel off the skin of potatoes, cut into small pieces and boil (200g) in 500ml of water, till they are easily penetrated by a glass rod. Filter through cheesecloth. Add dextrose to the filtrate. Dissolved agar in water and bring up to the required volume by the addition of water. Maintain pH upto 5.6 and then autoclaved at 15 lb pressure for 15 minutes.

Antifungal activity susceptibility test:

Antifungal activity test against pathogens were determined by Poison food technique (Grover and Moore, 1962; Mishra and Tiwari, 1992; Nene and Thapliyal, 2000). Potato dextrose agar (PDA) was used as a culture medium. From this required different concentration 100µl, 250µl, 500µl, 1000µl of extract was taken by sterilized pipette and mixed with 15 ml of cooled (45°C) molten PDA medium, poured into the sterile pertiplates and mixed well and

allowed to solidify at room temperature for thirty minutes. The plates were left overnight at room temperature to check for any contamination to appear. Inoculation was done at the center of each plate with 5 mm mycelium block for each fungus. The mycelium block was prepared with the help of cork borer from the growing area of a 5-7 days old fungal culture. The fungal disc was transferred aseptically at the center of each petriplate in an inverted position to get greater contact of the mycelium with the culture medium. Proper control (PDA without extract) serves as control. Antibiotics Amphotericine – B were used as positive control respectively (Mc Cutcheon *et al.,* 1994). The inoculated plates were incubated for 72 hours at temperature 26 ± 1°C. Diameter of fungal Colonies is recorded by measuring the two opposite circumference of the colony growth. The average of triplicate of measurements was taken as colony diameter of the fungus in millimeters. To ensure the sensitivity of pathogens to the plant extract. The percentage inhibition of mycelial growth of the test fungus was calculated by the following formula.

$$\% I = (C - T/ C) \times 100$$

Where, I = Percentage of inhibition, C = Diameter of fungal colony in control (mm),
T = Diameter of fungal colony in treatment (mm) (Rahman *et al.,* 2011).

STATISTICAL ANALYSIS:

Data analysis was done using SPSS statistical package for Windows (version 16.0, SPSS Inc). Data values were expressed as mean ± standard error. Statistical analysis was performed using One-way ANOVA, DMRT test using Duncan's univalent test was used for calculating for 5% level of significance ($p \leq 0.05$). Three-way ANOVA, DMRT test also using Duncan's multiple- range test was used for calculating for 1% level of significance ($p \leq 0.01$) and tests were further subjected to a post hoc test (Bonferroni test) to find the difference between the means.

3. RESULTS AND DISCUSSION

RESULTS

Results summarized in table 1. Preliminary phytochemical screening of leaves of *D. gangeticum* in **aqueous** extracts showed the presence of various phytocompounds *i.e.*, alkaloids, phenols, reducing sugar, saponins, tannins, terpenoids, flavonoids, fixed oils, anthraquinone while cardiac glycosides was absent. **Acetone** extracts showed the presence of alkaloids, phenols, reducing sugar, saponins, tannins, flavonoids, fixed oils, rest of the compounds terpenoids, cardiac glycosides, anthraquinone were absent. **Chloroform** extracts showed the presence of alkaloids, phenols, reducing sugar, saponins, flavonoids, fixed oils while rest of the compounds tannins, terpenoids, cardiac glycoside, anthraquinone were absent. However, **Petroleum ether** extracts showed the presence of alkaloids, phenols, reducing sugar, saponins, flavonoids, fixed oils, while rest of the compounds tannins, terpenoids, cardiac glycoside, anthraquinone were absent. These findings were similar to those of previous reports in aqueous extract of leaves showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, tannins, saponins, volatile oils whereas amino acids, anthroquinones, cardiac glycosides, phenols, steroids and terpenoids were found to be absent. Alkaloids, terpenoids, tannins, volatile oils were found to be present in petroleum ether extract whereas amino acids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, saponins, phenols, steroids were found to be absent. Alkaloids, amino acids, carbohydrates, flavonoids, glycosides, tannin, saponins, volatile oils, phenols, steroids were present in chloroform extract whereas anthraquinones, cardiac glycoside, terpenoids were found to be absent (Avasthi *et al.,* 1995; Mishra *et al.,* 2005; Ning *et al.,* 2009; Vijayalaksmi *et al.,* 2011; Vaghela *et al.,* 2012; Bhattacharjee 2013; Haridas *et al.,* 2016; Vepal *et al.,* 2016; Mutyala Naidu L. and Aniel Kumar O. 2016; Srivastava and Srivastava., 2018).

Results summarized in table 2. (Photoplate no 1.) In present study, aqueous leaf extracts of *D. gangeticum* showed maximum growth inhibition % at 1000µl conc. against *C. albicans* (62.96 ± 1.85) which was nearest to the value of the standard antibiotics Amphotericine- B (72.22 ± 0) while with the other solvents acetone, chloroform and petroleum ether extract showed moderate sensitivity. Minimum growth inhibition % was recorded (48.14 ± 4.90) at lower concentration of 100µl in aqueous leaf extracts. One-way ANOVA summary ($F= 4.339$; $df= 19,40$; $p \leq 0.05$) reported significant difference value between the solvent and growth inhibition percent graphically represented in fig 1. Chloroform leaf extracts showed maximum growth inhibition % at 1000µl conc. on *C. lunata* (70.7 ± 8.26) which was nearest to the value of the standard antibiotics Amphotericine- B (84.41 ± 0.53) while with acetone, aqueous and petroleum ether extract showed moderate sensitivity. Minimum growth inhibition % was recorded (48.48 ± 10.64) at lower concentration 100µl in chloroform leaf extracts. One-way ANOVA summary ($F= 5.6$; $df= 19,40$; $p \leq 0.05$) reported significant difference value graphically represented in fig 2. Acetone leaf extracts showed maximum growth inhibition % at 1000µl conc. on *M. gypseum* (68.42 ± 0) which was nearest to the value of the

standard antibiotics Amphotericine- B (71.93 ± 1.75) while with chloroform, aqueous and petroleum ether extract showed moderate sensitivity. Minimum growth inhibition % (57.89 ± 0) at lower concentration 100µl in chloroform leaf extracts. One-way ANOVA summary (F= 2.567; df= 19,40; p≤0.05) reported no significant difference values graphically represented in fig 3. Chloroform leaf extracts showed maximum growth inhibition % at 1000µl conc. on *C. oxysporum* (84.25 ± 4.62) which was more than the value of the standard antibiotics Amphotericine- B (82.18 ± 1.51) while with aqueous, acetone and petroleum ether extract showed moderate sensitivity. Minimum growth inhibition % (80.55 ± 5.61) was recorded at lower concentration 100µl in chloroform leaf extracts. One-way ANOVA summary (F= 1.879; df= 19,40; p≤0.05) reported significant difference values graphically represented in fig 4. Acetone leaf extracts showed maximum growth inhibition % at 1000µl conc. on *T. viride* (87.11 ± 0.44) which was nearest to the value of the standard antibiotics Amphotericine- B (88.89 ± 0.44) while with petroleum ether, aqueous and chloroform extract showed moderate sensitivity. Minimum growth inhibition % (73.77 ± 1.93) was recorded at lower concentration 100µl in acetone leaf extracts. One-way ANOVA summary (F= 3.706; df= 19,40; p≤0.05) reported significant difference value graphically represented in fig 5. Acetone leaf extracts showed maximum growth inhibition % at 1000µl conc. on *A. niger* (91.55 ± 0.44) which was more than the value of the standard antibiotics Amphotericine- B (87.55 ± 1.77) while with chloroform, aqueous and petroleum ether extract showed moderate sensitivity. Minimum growth inhibition % was recorded (83.55 ± 1.17) at lower concentration 100µl in acetone leaf extracts. One-way ANOVA summary (F= 19.79; df= 19,40; p≤0.05) thus, the value showed significant difference is graphically represented in fig 6.

Results summarized in table 3. Each value represents the mean of three measurements ± std. error mean from three petriplates having similar values of crude plant extracts. Three-Way ANOVA analysis was carried out to determine effect of different fungal strains, different solvents (aqueous, acetone, chloroform, petroleum ether), different concentrations (100, 250, 500, 1000µl, Amphotericine- B) of *D. gangeticum*(L.) DC. plant on growth inhibition % of *C. albicans*, *C. lunata*, *M. gypseum*, *C. oxysporum*, *T. viride*, *A. niger*. Three-way ANOVA summary (F= 0.171; df= 60,240; p=1) thus, the value showed no significant difference p≤0.01 (Based on Duncan's multiple-range test, DMRT).

D. gangeticum (L.) DC. in acetone extracts showed highest antifungal activity against *A. niger* (91.55 ± 0.44) followed by *T. viride* (87.11 ± 0.44), *C. oxysporum* (84.25 ± 4.62), *C. lunata* (70.7 ± 8.26), *M. gypseum* (68.42 ± 0), *C. albicans* (62.96 ± 1.85). Acetone extracts showed best activity and least activity was observed in aqueous extracts. Similar works were performed by Avasthi *et al.,* (2010) out of eight plant materials used, five showed significant antifungal activity against *Aspergillus niger*. *Syzygium aromaticum* and *Allium sativum* showed 100% inhibition of mycelia growth at 20% concentration. In *Cinnamomum zeylanicum*, *Trachy spermumammi* and *Piper nigrum* a moderate inhibition (52.4%, 48.93%, 46.2%) was reported whereas, no inhibition was in other plant products. Hemlal and subban (2012) also reported the antimicrobial activity of *Pseudarthria viscid* Wight and Arn and *Desmodium gangeticum* (Linn) DC. using poison food technique. Gautam *et al.,* (2015) reported *Althaea officinalis* Linn. seed extracts and essential oil extract exhibits the percentage inhibition of 41.28% by essential oil against *A. niger*, aqueous extracts with 36.27% and Methanolic extract with 23.89% of inhibition respectively and the control mycelial growth diameter was (33.6 ± 0.57 – 37.6 ± 0.28 mm). Kumar and Tyagi (2013) reported the antifungal activity of *Bergenia stracheyi* against all the six fungal species *A. alternate*, *A. niger*, *C. gloeosporioides*, *F. oxysporum*, *G.lucidum* and *R. solani*.

CONCLUSION

From this study we concluded that *D. gangeticum* (L.) DC. Consist several phytochemicals and have potential antifungal property against selected fungal strains. Further research should be conducted for isolation and characterization of bioactive compounds responsible for antifungal activity must be performed using TLC and HPLC analysis for more effective outcomes. As medicinal plants have made significant contribution towards human health to replace synthetic drugs with low cost and less side effects, yet minimal scientific study has been carried out in the area of medicinal plants having antifungal properties. Lot of medicinal plants contained various healing properties and their active principles need to be investigation. Therefore, further study is been recommended on antifungal susceptibility tests.

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REFERENCE

Avasthi, B. K. and Tewari, J. D. (1955). Chemical investigation of *Desmodium gangeticum*. II. Chemical constitution of the lactone. *J. Am. Pharm. Assoc* (Baltim), **44(10):** 628-629.

Avasthi, S.; Gautam, A. K.; Bhadauria, R. (2010). Antifungal activity of plants products against *Aspergillus Niger*: A potential application in the control of a spoilage fungus. *Biological Forum – An International Journal*, **2(1):** 53-55.

Bhattacharjee, I.; Chatterjee, SK.; Chandra, G. (2010). Isolation and identification of antibacterial components in seed extracts of *Argemone Mexicana* L. (Papavetaceae). *Asian Pacific J Trop Med*, **3(7):** 547-551.

Bhattacharjee, A.; Shashidhara, S C. and Saha, S. (2013). Phytochemical and ethno-pharmacological profile of *Desmodium gangeticum* (L.) DC.: A review.. *International Journal of Biomedical Research*, **4(10):** 507-515.

Doherty, Vf.; Olaniran, OO.; Kanife, Uc. (2010). Antimicrobial activities of *Aframomum Melegueta* (Alligator Pepper). *International Journal of Biology*, **2(2):** 126-131.

Gautam, S.S.; Kumar, N.S.; Chauhan, R. (2015). Antimicrobial efficacy of *Althaea officinalis* Linn. seed extracts and essential oil against respiratory tract pathogens. *Journal of Applied Pharmaceutical Science*, **5(09):** 115-119.

Grover, RK. and Moore, JD. (1962). Taximetric studies of fungicides against brown rot organism- *Sclerotinia fructicola* and *S. laxa*. *Phytopathology*, **52**: 876-880.

Harbone, JB. (1973). Phytochemical Methods. Chapman and Hall, Ltd., London, 49-188.

Haridas, H.; George, C S.; Krishnan, D.; Jose, A. and Jayachandran, T P. (2016). A Review of Pharmacognostic and Phytochemical evaluation of plant *Desmodium gangeticum* (L.) DC. *World Journal of Pharmaceutical Research*, **5(4):** 463-471.

Hemlal, H. and Subban, R. (2012). GC-MS, HPTLC and antimicrobial analysis of root extracts of *Pseudarthria viscid* Wight and Arn and *Desmodium gangeticum* (L.) DC. *International Research Journal of Biological Sciences*, **1(5):** 57-65.

Horowitz, S. v. (1984). Counrt of Appeals of California, First Appellate District, Division Three.

Http://Envis .Frlht.Org - Envis Centre on Conservation of Medicinal Plants, Frlht, Banglore Http://Frlhtenvis.Nic.In (22 September 2010).

Kosalge, Sb. and Fursule, Ra. (2009). Investigation of Ethnomedicinal claims of some plants used by Tribals of Satpuda Hills in India. *Journal of Ethnopharmacology*, **121(3):** 456-461.

Kumar, V. and Tyagi, D. (2013). Antifungal activity evaluation of different extracts of *Bergenia stracheyi*. *International Journal of Current Microbiology and Applied Sciences*, **2(7):** 69-78.

Mahajon, B.; Deepak, M.; Rema Shree A.B.; Remadevi, R. (2015). Comparitive phytochemical analysis of different parts of Sthiraa- *Desmodium gangeticum* (L.) DC. *World Journal of Pharmaceutical Research*, **4(8):** 1821-1828.

Mc Cutcheon, A. R.; Ellis, S. M.; Hancock, R. E. W.; Ower.ss, G. H. N. T. (1994). Antifungal screening of medicinal plants of British Columbian Native People. *J. Ethnopharmacol*, **44:** 157-169.

Mishra, M. and Tiwari, SN. (1992). Toxicity of *Polyalthia longifolia* against fungal pathogens of rice. *Indian Phytopath*, **45:** 56-61.

Mishra, PK.; Singh, N.; Ahmed, G.; Dube, A.; Maurya, R. (2005). Glycolopids and other Constituents from *Desmodium gangeticum* with antileishmania and immunomodulatory activities. *Bioorg Med Chem Lett*, **15(20):** 4543-4546.

Mutyala Naidu L. and Aniel Kumar O. (2016). Amtimicrobial activity and phytochemicals constituents of *Desmodium gangeticum* leaves. *International Research Journal of Agriculture and Food Sciences*, **1(3):** 44-52.

Nene, Y. and Thapliyal, L. (2000). Poisoned food technique of fungicides in plant disease control (3rd eds). Oxford and IBH publishing Company, New Delhi.

Ning, G.; Tianhua, L.; Xin, Y.; He, P. (2009). Constitutes in *Desmodium blandum* and their antitumor activity. *Chin Trad Herb* Drug, **40:** 852-856.

Pullaiah, T. (2012). Medicinal Plants In India Volume 1regency Publications New Delhi. ISBN: 82-87498-57-9.

Rahman, MA.; Chakma, JS.; Islam, S.; Ahmed, NU. (2011). Evaluation of antioxidant, antibacterial, antifungal and cytotoxic effects of *Clausena suffruticosa* ethanolic root extract. *J Appl Pharma*, **1(5):** 90-95.

Srivastava, P. and Srivaastava, G. (2018). Pharmacological and phytochemical screening of *Desmodium gangeticum* and *Moringa oleifera*. *Research Journal of Chemistry and Environment*, **22(5):** 6-10.

Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, 191-289.

Tabuti, Jr.; Lye, Ka.; Dhillion, Ss. (2003). Tradition Herbal Drugs Of Bulamogi, Uganda: Plants, Use And Administration. *Journal of Ethnophermacology*, **88(1):** 19-44.

Trease, G. E. and Evans, W. C. (1989). "Pharmacognosy". *Thirteenth Edition Bailliere*, London. 882.

Vaghela, BD.; Patel, BR.; Pandya, PN. (2012). A comparative pharmacognostical profile of *Desmodium gangeticum* DC. and *Desmodium laxiflorum* DC. Ayu, **33:** 552-526.

Vedpal, S.P. Dhanabal; Dhamodaran, P.; Chaitnya, M V N L.; Duraiswamy, B.; Unni, J. and Srivastava, N. (2016). Ethnopharmacological and Phytochemical profile of three potent Desmodium Species: *Desmodium gangeticum* (L.) Dc, *Desmodium triflorum* Linn and *Desmodium triquetrum* Linn.. *Journal of Chemical and Pharmaceutical Research*, **8(7):** 91-97.

Venkatachalam, U. and Muthukrishnan, S. (2012). Free radical scavenging activity of ethanolic extracts of *Desmodium gangeticum*. *Journal of Acute Medicine*, **2(2012):** 36-42.

Vijayalakshmi, G.; Deepti, K.; Arjuna Rao, P. V. and Lakshmi, KVSS. (2011). Phytochemical evaluation and antimicrobial activity of crude extracts of *Desmodium gangeticum* DC. *Journal of Pharmacy Research*, **4(7):** 2335-2337.

Yadav, RNS. and Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology*, **3(12):** 10-14.

WWW.IUCN.org.

C. albicans	*C. lunata*	*M. gypseum*	*C. oxysporum*	*T. viride*	*A. niger*
					
					

Photoplate 1. *D. gangeticum* (L.) DC. different solvent leaf extracts showing growth inhibition percent (%) against six fungal strains.

Table 1. Preliminary phytochemical screening of leaf extracts of *D. gangeticum* (L.) DC.

Chemical constituents	Test Performed	Observation	Inference			
			AQ	AC	CH	PE
Alkaloids	Mayer's test	Presence of turbidity	++	++	+	+
Phenols	Ferric chloride test	Bluish green color	+	++	+	+
Reducing sugar	Fehling's test	Brick red color	++	++	++	++
Saponin	Froth test	Frothing persist 15 mins	+	+	+	+
Tannin	Ferric chloride test	Dark blue or greenish grey Cream ppt	+	++	-	-
Terpenoid	Salkowski's test	Bluish-green color at interphase reddish color	+++	-	-	-
Flavonoids	Ammonia reduction test	Yellow colour	+	+	+	+
Fixed oils	Filter paper test	Oil stain develops	+++	+++	+++	+++
Cardiac glycosides	Keller- Killani test	Violet ring below brown ring interphase	-	-	-	-
Anthraquinone	Borntrager's test	Deep red or Pink color of aqueous layer	++	-	-	-

AQ; aqueous, AC; acetone, CH; chloroform, PE; petroleum ether extracts

'+++' relatively a strong presence; '++' relatively moderate presence; '+' relatively low presence, '-' indicates absence.

Table 2. Effect of different concentrations of crude extracts from *D. gangeticum* (L.) DC. on growth inhibition % against *C. albicans, C. lunata, M. gypseum, C. oxysporum, T. viride, A. niger*. Each value represents the mean of three measurements ± std. error from three petriplates having a similar values of crude plant extracts. Significant difference at the 5% level between values obtained under control and the different concentration of extracts treatments (P≤0.05), according to DMRT.

Micro organism	Conc. (in µl)	Growth inhibition % (Mean (mm) ± Std. error)				
		AQ	AC	CH	PE	Ampho- B
C. albicans	**100µl**	48.14 ± 4.90^{efgh}	46.29 ± 1.85^{fgh}	44.44 ± 5.55^{gh}	42.59 ± 3.70^{h}	57.4 ± 1.85^{bcdef}
	250µl	53.7 ± 4.9^{cdefgh}	50 ± 0^{defgh}	48.14 ± 4.90^{efgh}	46.29 ± 3.70^{fgh}	62.96 ± 1.85^{abc}
	500µl	59.26 ± 1.85^{bcde}	55.55 ± 0^{bcdefg}	53.7 ± 4.9^{cdefgh}	53.7 ± 4.9^{cdefgh}	66.66 ± 0^{ab}
	1000µl	62.96 ± 1.85^{abc}	61.11 ± 0^{abcd}	57.4 ± 6.67^{bcdef}	57.4 ± 6.67^{bcdef}	72.22 ± 0^{a}
C. lunata	**100µl**	38.38 ± 14.56^{def}	39.39 ± 8.01^{def}	48.48 ± 10.64^{cd}	21.21 ± 4.62^{f}	77.95 ± 0.54^{ab}
	250µl	47.47 ± 10.54^{cde}	50.5 ± 3.64^{cd}	51.51 ± 14.31^{cd}	23.23 ± 5.62^{ef}	80.64 ± 0^{a}
	500µl	52.52 ± 7.28^{cd}	55.55 ± 1.01^{bcd}	60.6 ± 14.31^{abcd}	37.37 ± 4.04^{def}	81.71 ± 0.53^{a}
	1000µl	55.55 ± 6.14^{bcd}	62.62 ± 2.67^{abcd}	70.7 ± 8.26^{abc}	53.53 ± 2.67^{bcd}	84.41 ± 0.53^{a}
M. gypseum	**100µl**	38.59 ± 4.64^{cde}	57.89 ± 0^{abcd}	47.36 ± 13.24^{abcde}	28.07 ± 12.65^{e}	54.38 ± 4.64^{abcd}
	250µl	54.38 ± 7.02^{abcd}	61.4 ± 1.75^{abcd}	50.87 ± 12.28^{abcde}	36.84 ± 13.24^{de}	61.4 ± 1.75^{abcd}
	500µl	59.65 ± 4.64^{abcd}	66.66 ± 1.75^{ab}	63.16 ± 5.26^{abc}	45.61 ± 12.28^{bcde}	68.42 ± 0^{ab}
	1000µl	63.15 ± 3.04^{abc}	68.42 ± 0^{ab}	64.91 ± 3.51^{ab}	49.12 ± 9.76^{abcde}	71.93 ± 1.75^{a}
C. oxysporum	**100µl**	61.11 ± 18.75^{abcd}	41.66 ± 21.62^{bcd}	80.55 ± 5.61^{ab}	29.16 ± 5.25^{d}	68.32 ± 1.50^{abcd}
	250µl	62.96 ± 19.67^{abcd}	53.24 ± 17.50^{abcd}	82.87 ± 5.33^{ab}	33.79 ± 7.58^{cd}	72.98 ± 0.57^{abc}
	500µl	66.66 ± 18.12^{abcd}	62.96 ± 12.98^{abcd}	83.33 ± 4.87^{a}	46.76 ± 6.82^{abcd}	77.58 ± 0.99^{ab}
	1000µl	68.52 ± 18.98^{abcd}	67.59 ± 11.9^{abcd}	84.25 ± 4.62^{a}	60.64 ± 10.82^{abcd}	82.18 ± 1.51^{ab}
T. viride	**100µl**	49.77 ± 23.60^{abcd}	73.77 ± 1.93^{a}	18.66 ± 8.87^{d}	48 ± 6.3^{abcd}	81.77 ± 0.88^{a}

	250µl	51.11 ± 22.96^{abcd}	77.77 ± 1.93^{a}	20.88 ± 13.25^{cd}	60 ± 2.77^{abc}	84.44 ± 0.44^{a}
	500µl	53.91 ± 23.72^{abcd}	83.11 ± 0.44^{a}	28.88 ± 11.75^{bcd}	66.66 ± 3.84^{ab}	87.11 ± 0.44^{a}
	1000µl	58.22 ± 24.45^{abcd}	87.11 ± 0.44^{a}	31.11 ± 12.37^{bcd}	76 ± 1.53^{a}	88.89 ± 0.44^{a}
A. niger	**100µl**	63.11 ± 4.23^{ef}	83.55 ± 1.17^{abcd}	60 ± 1.53^{f}	16.89 ± 11.58^{h}	64.88 ± 1.17^{ef}
	250µl	67.11 ± 2.70^{def}	85.77 ± 0.44^{abc}	69.33 ± 2.03^{cdef}	16.89 ± 11.58^{h}	79.55 ± 0.44^{abcde}
	500µl	70.22 ± 1.78^{bcdef}	87.11 ± 0.44^{ab}	68.44 ± 1.17^{cdef}	20 ± 12.02^{h}	85.77 ± 0.88^{abc}
	1000µl	73.33 ± 0^{bcdef}	91.55 ± 0.44^{a}	76.89 ± 3.55^{abcdef}	39.55 ± 10.04^{g}	87.55 ± 1.77^{ab}

Table 3. Summary Three–way ANOVA analysis on growth inhibition (%) of *D. gangeticum* (L.) DC.

Factors	Factors (F)	Degree of freedom (df)	P
Fungus	6.526	5,240	≤0.01
Solvent	48.183	4,240	≤0.01
Concentration	20.815	3,240	≤0.01
Fungus*Solvent	10.754	20,240	≤0.01
Fungus*Concentration	0.136	15,240	1
Solvent*Concentration	0.456	12,240	0.938
Fungus*Solvent*Concentration	0.171	60,240	1

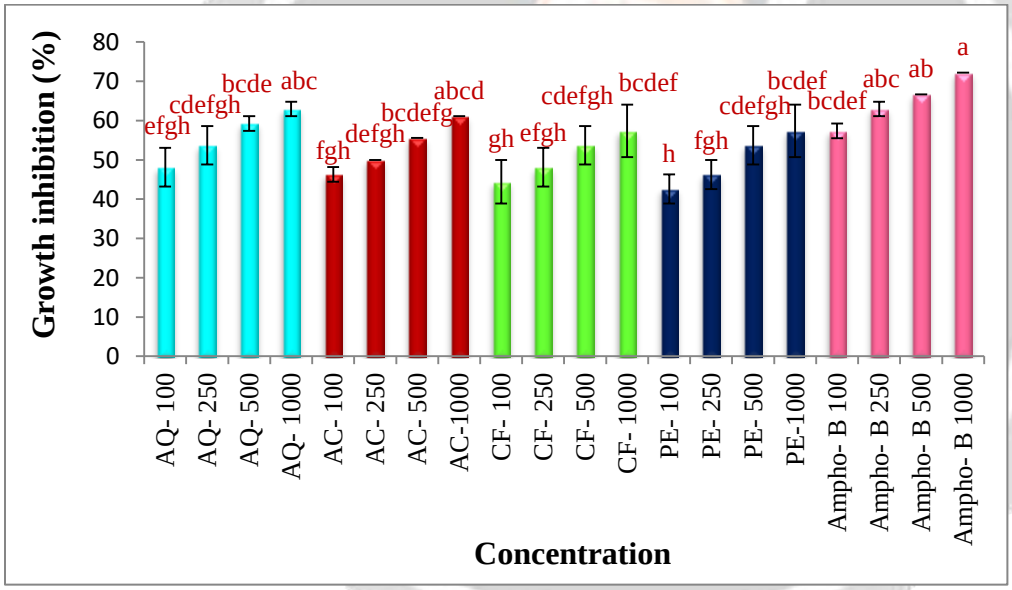

Figure 3.1.1.1. Effect of *D. gangeticum* (L.) DC. crude leaf extracts on *Candida albicans*: ANOVA summary (F= 4.339; df= 19,40; p≤0.05). Means having similar alphabets are not statically significant from each other at p≤0.05 (Based on Duncan's test, DMRT).

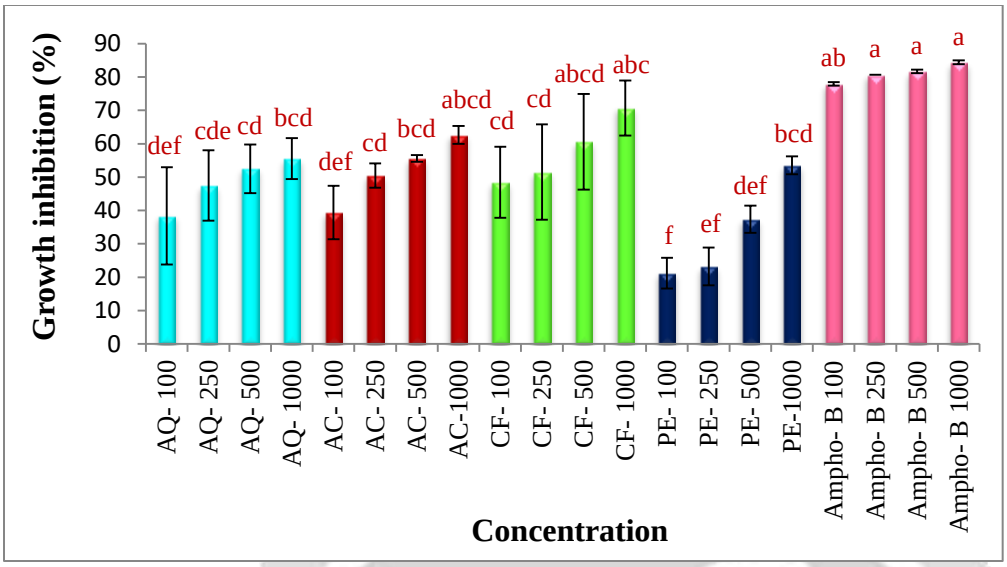

Figure 3.1.1.2. Effect of *D. gangeticum* (L.) DC. crude leaf extracts on *Curvularia lunata*: ANOVA summary (F= 5.6; df= 19,40; p≤0.05). Means having similar alphabets are not statically significant from each other at p≤0.05 (Based on Duncan's test, DMRT).

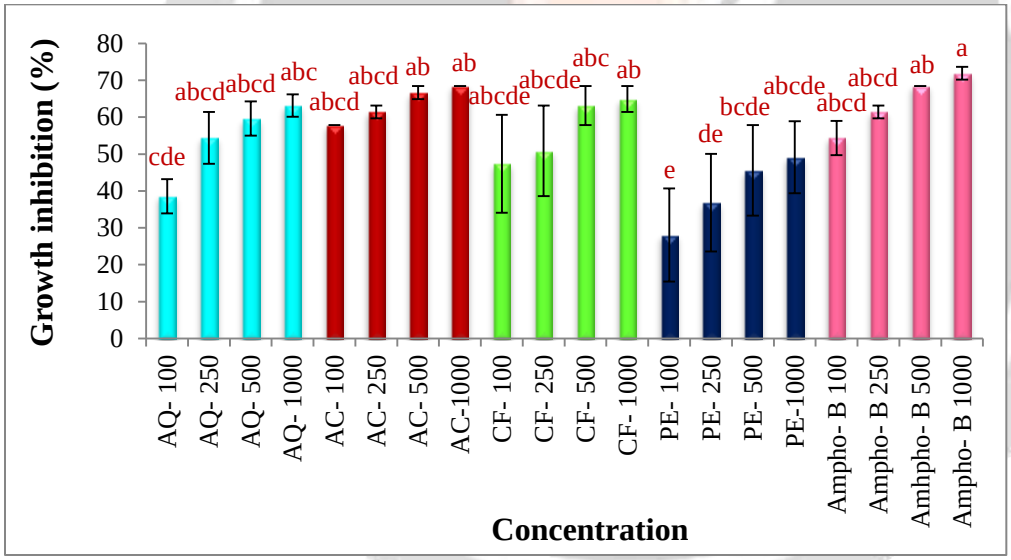

Figure 3.1.1.3. Effect of *D. gangeticum* (L.) DC. crude leaf extracts on *Microsporum gypseum*: ANOVA summary (F= 2.567; df= 19,40; p≤0.05). Means having similar alphabets are not statically significant from each other at p≤0.05 (Based on Duncan's test, DMRT).

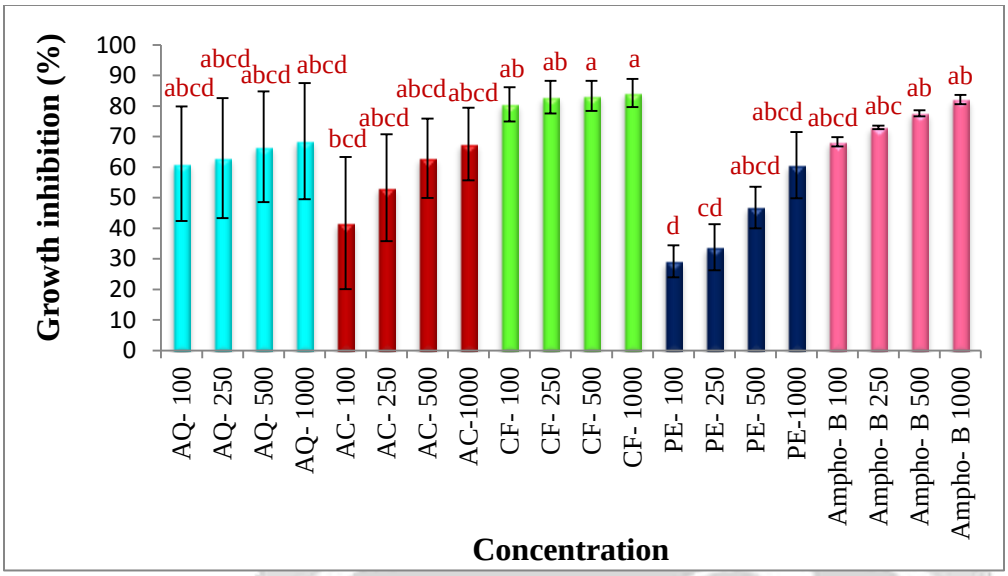

Figure 3.1.1.4. Effect of *D. gangeticum* (L.) DC. crude leaf extracts on *Cladosporium oxysporum*: ANOVA summary (F= 1.879; df= 19,40; p≤0.05). Means having similar alphabets are not statically significant from each other at p≤0.05 (Based on Duncan's test, DMRT).

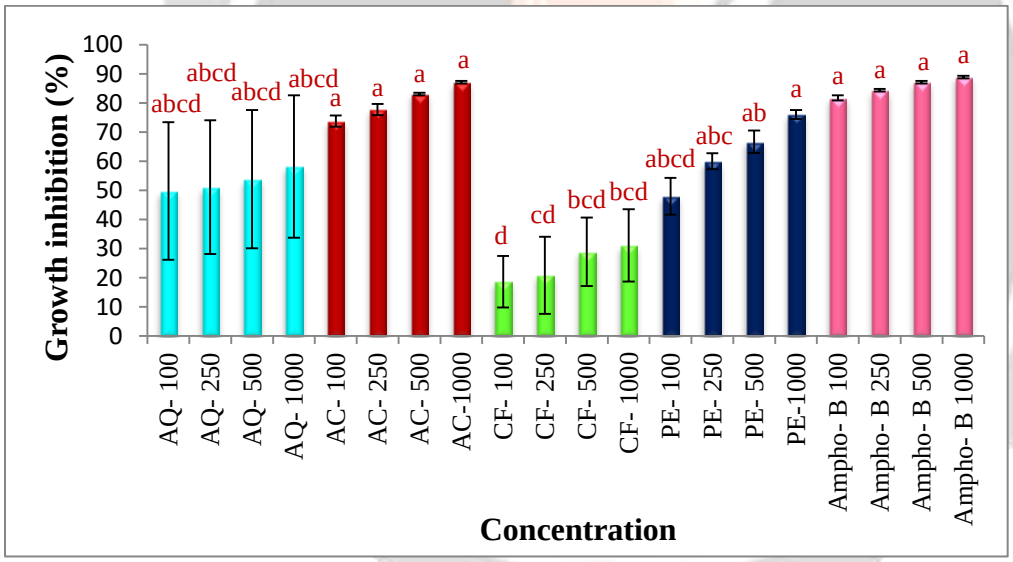

Figure 3.1.1.5. Effect of *D. gangeticum* (L.) DC. crude leaf extracts on *Trichoderma viride*: ANOVA summary (F= 3.706; df= 19,40; p≤0.05). Means having similar alphabets are not statically significant from each other at p≤0.05 (Based on Duncan's test, DMRT).

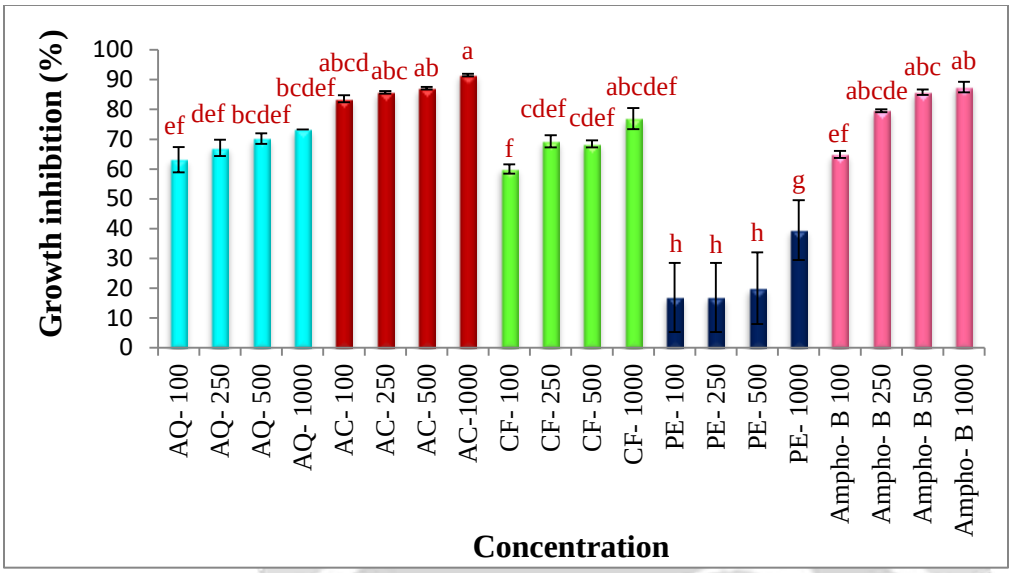

Figure 3.1.1.6. Effect of *D. gangeticum* (L.) DC. crude leaf extracts on *Aspergillus niger*: ANOVA summary (F= 19.79; df= 19,40; p≤0.05). Means having similar alphabets are not statically significant from each other at p≤0.05 (Based on Duncan's test, DMRT).