STUDY OF PHYTO CHEMICAL SCREENING AND IN-VITRO ANTI OXIDANT ACTIVITY OF DELONIX REGIA LEAVES EXTRACT

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ABSTRACT

Objective:- phytoconstituents can play an important role in traditional medicines and are under investigation for antibacterial, antimicrobial, and other pharmaceutical functions. The present study is to explore phytoconstituents present in the ethyl acetate extract of Delonix regia (hook) leaves.

Methods:-95% of methanol crude extract was further partitioned with different solvents. The ethyl acetate soluble part was subjected to column chromatography and eluted with solvent mixtures of increasing polarity. The structures of these isolated compounds were identified on the basis of spectral analysis. I.e. FT-IR, NMR, UV, VISIBLE, MASS, etc.

Result:-95% of methanolic extract of the leaves of Delonix regia the isolation of sterols and glucoside namely,stigmasten-diol-3-o-glucoside,12,15-Dihydroxy-chol-8-en-24-oic acid-3-oxy-6-acetyl-glucoside and sodium, potassium adduct of 12,15-Dihydroxy-5-chol-9-en-24-oic-acid-3-oxy-rhamnosyl-rhamnosyl-rhamnoside, one flavonol, name kaempferol.

Conclusion:-On the isolation and identification of chemical constituents from Delonix regia leaves. These molecules would be useful in the treating hyperlipid in patients.

Keywords:- Delonix regia, Kaempferol, Stigmasten-diol-3-0-glucoside,sterol,flavanoid.

INTRODUCTION:- Delonix regia is evergreen but in some dry areas it sheds its leaves during the drought. The tree is known for its flowers and in spring as well as in summer season the tree is covered

with lively clusters of flame red petals, which covers the tree from May to June (Aridus, 2004). The height of the plant reaches up to 5 to 8 m (Warren, 2013), but its elegant wide spreading umbrella like canopy which can be wider than its height. The appearance of the leaves is feathery and bi-pinnately compound with a characteristic colour which is light but bright green and arrangement is alternate. The leaves of the plant are delicate and are fern like, composed of small individual leaflets, which fold up at the onset of dusk. They are doubly pinnate, oblong and have entire margin. Each leaf is 30 to 50 cm long and has 20 to 40 pairs of primary leaflets or pinnae on it, and each of these is further divided into 10 to 20 pairs of secondary leaflets or pinnules. The D. regia provides fullest flowering and best growth when planted in full sun location (Edward et. al., 1993). The petals of the flower grow in corymbs along and at the ends of branches. The colour of the flower is orange or red and comprises of five petals out of which four are spoons shaped.

MATERIALS AND METHODS:-

Collection, identification and preparation of plant

The leaves of the plant Delonix regia collected from Bhopal district in the month of may 2017 and authenticated by DR mukta shrivastava ,Govt M.L.B. COLLEGE Bhopal.madhya Pradesh.The leaves were cleaned and dried in the shade and crushed in to powder.

Extraction and isolation:-

The powered sample was extracted by soxhlet extraction method. Use methanol,and petroleum ether one after the other,check for purity using TLC. Before futher purification using HPLC .

The initial weight of 30 gms of the dried petals of Delonix regia was taken in 100 ml of methanol. The percentage yield of 6.16 percent was obtained in the methanolic extract of petals of D. regia, whereas 6.68 percent in the methanolic extract of leaves of D. regia. The percentage yield of extracts of petals and leaves of D. regia in methanol.

Phyto-chemical Analysis of Leaves Extract of D. regia:-

Phyto-chemical analysis involves the qualitative analysis of herbal plant exracts. The preliminary qualitative tests have been attempted in Delonix regia leaves to find out the presence or absence of certain bio active compounds. The chemical tests were carried out on the crude methanolic extract using standard procedures to identify the active constituents.The crude methanolic extracts of leaves of D. regia were evaluated qualitatively to analyze the presence of secondary metabolites. the secondary metabolites found in crude methanolic extract of leaves were found to be anthraquinones, flavonoids, glycosides, steroids, tannins and terpenoids. The D. regia crude methanolic extract of leaves showed positive result to the different phyto-chemical tests indicating the presence a number of phyto-constituents. The presence of alkaloid was analyzed in methanolic extract of leaves with Wagner’s method. The presence of reddish brown colored precipitate indicates the presence of alkaloids. The absence of reddish brown colored precipitate in the methanolic leaves extract, indicating the absence of alkaloids in them.The Borntrager’s test was performed for the analysis of anthraquinones in the

methanolic extract of leaves. The formation of rose pink colour in plant extract confirmed the presence of anthraquinones. the methanolic leaves extract when tested using the Borntrager's test confirmed the appearance of pink colour indicating the presence of anthraquinones in the methanolic extract of leaves.The presence of flavonoids in the crude plant extract is determined quantitatively by the appearance of yellow colour. When the methanolic extract of leaves of D. regia was evaluated using this test showed the appearance of yellow colour indicates the presence of flavonoids .The presence of glycosides in the D. regia methanolic leaves extract was evaluated using the Fehling's test. The brick red precipitate formation indicates the presence of glycosides. D. regia leaves extract showed the presence of brick red precipitate thus confirming the presence of glycosides in crude extract.Similarly the presence of saponins in the plant extracts evaluated using a frothing test. The formation of froth confirmed the presence of saponins. The D. regia leaves extracts did not show the appearance of froth indicating the absence of saponins .The D. regia crude methanolic leaves extracts were also evaluated for the presence of steroids by using the Salkwoski test. The change of colour from violet to blue indicates a positive result. The crude methanolic leaves extracts showed the change of colour from violet to blue thus confirming the presence of steroids in the extract.The crude leaves extract were further tested for the presence of tannins by using ferric chloride test. The occurrence of blue black precipitate indicates the presence of tannins. The D. regia methanolic leavesl extract showed the formation of blue black precipitate thus confirming the presence of tannins.Similarly, Salkowski test was also performed to evaluate the presence of terpenoids in D. regia curde methanolic extract of leaves . The formation of reddish brown colour indicates the presence of terpenoid.

Anti-oxidant Activities of Leaves Extract of Delonix regia:-

Anti-oxidant activity of methanolic leaves extract of D. regia was determined in vitro by using a number of assays , DPPH free radical scavenging activity, H_2O_2 radical scavenging activity and total anti-oxidant capacity method.The hydrogen peroxide is not a strong oxidizing agent. It can cause inactivation of some enzymes directly, by oxidation of the thiol (-SH) groups. It can easily cross cell membrane rapidly. Once reached inside the cell, H_2O_2 can possibly reacts with Fe^{2+} and possibly Cu^{2+} to form hydroxyl radical. The formation of hydroxyl radical is the initial step of the formation of many toxic effects (Miller et. al., 1993). It is therefore very important and necessary for the cells to control the production of hydrogen peroxide which was built up in vivo. The scavenging of H_2O_2 attributes to their phenolic content which donate electrons to H_2O_2, thus was neutralizing it to water (Halliwell and Gutteridge, 1985). The ability of the extract to effectively scavenge hydrogen peroxide were determined according to the method done by Ruch et. al., (1989) where they were compared with BHT. The D. regia methanolic extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner. The leaves extracts exhibited 68.84 ± 0.45 and 1.15 ± 0.381 percent inhibition respectively, at the concentration of 1000 µg/ml and 1.95 µg/ml by hydrogen peroxide scavenging activity. On the other hand, at the same concentration butylated hydroxy toluene exhibited 77.03 ± 0.128 and 4.14 ± 0.128 percent inhibition respectively. The IC50 value of D. regia leaves extract was found to be 326.43 ± 5.773 µg/ml whereas the IC50 value of BHT was found to be 26.16 ± 0.351 µg/ml. 4.9.4 Anti-oxidant Activity by DPPH (2, 2 – Diphenyl – 1- Picryl Hydrazyl) Radical Scavenging Assay The DPPH radical scavenging assay showed the ability of the extracts and the standard (BHT) to scavenge DPPH free radicals. The DPPH radical exists

naturally in deep violet colour but when reacts with anti-oxidant it turn into a yellow coloured diphenyl picryl hydrazine. The degree of discoloration indicates the radical-scavenging potential of the anti-oxidant (Tirzitis and Bartosz, 2010). The methanolic leaves extracts exhibited 64.77 ± 0.456 and 1.03 ± 0.222 percent inhibition, at the concentration of 1000 µg/ml and 1.95 µg/ml respectively, whereas the percentage inhibition values of BHT were found to be 73.03 ± 0.128 and 12.59 ± 0.128 percent, at the concentration of 1000 µg/ml and 1.95 µg/ml respectively. . The IC50 value of D. regia leaves extract was found to be 332.2 ± 3.983 µg/ml. Whereas IC50 value of butylated hydroxytoluene was 43.40 ± 1.307 µg/ml. 4.9.5 Total Anti-oxidant Capacity by Phosphomolybdenum Method The total anti-oxidant capacity of the methanolic crude plant extracts and standard (BHT) were determined by using the method of phosphomolybdenum. The higher absorbance value indicates the greater anti-oxidant activity. The total anti-oxidant capacity of plant extracts were measured at 695nm, spectrophotometrically using total anti-oxidant activity by phosphomolybdenum method. This method is based on the reduction of Mo (IV) to Mo (V) by the test sample and the formation of green phosphate/Mo (V) compounds (Abbasi et. al., 2010). A high absorbance value of the sample indicates its strong anti-oxidant activity. The total anti-oxidant capacity may be contributed due to their chemical composition and phenolic acid content. The methanolic leaves extracts exhibited 50.37 ± 0.189 and 0.25 ± 0.109 percent inhibition, at the concentration of 1000 µg/ml and 1.95 µg/ml respectively. The percentage inhibition values of standard (BHT) were found to be 77.12 ± 0.322 and 20.10 ± 0.207 percent, at the concentration of 1000 µg/ml and 1.95 µg/ml respectively. The high percentage inhibition indicates high scavenging activity of the plant extract. The IC50 value of D. regia leaves extract was found to be 976.84 ± 13.140 µg/ml, whereas the IC50 value of butylated hydroxytoluene was 124.25 ± 3.04 µg/ml. 4.10 IC50 Value of Different Anti-oxidant Activity The IC50 values of the methanolic extracts were calculated based on the results of different anti-oxidant assay conducted by DPPH, total anti-oxidant assay and hydrogen peroxide method. Delonix regia 108 Table No. 4.3: The different concentrations of extracts used from 1000 to 1.95 µg/ml.

RESULTS:-the present study shows the phytochemical screening and in vitro anti oxidant assay of methanolic extract of Delonix regia . the total phenolic content (TPC) by MEOH is 133.300 and by ethyl acetate (EtOAC) IS 98.300. Whereas total flavonoid content(TFC) by MEOH is 268.200 and by ethyl acetate(EtOAC) is 158.800.

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