**ABSTRACT**

The present work was aimed at phytochemical and pharmacological investigation of *Amaranthus spinosus* (leaves), *Amaranthus caudatus* (whole plant) and *Amaranthus viridis* (whole plant), family Amaranthaceae. *In vitro* antioxidant, alpha amylase inhibition, non-enzymatic hemoglycosylation, analgesic, antipyretic, anthelmintic and antimicrobial effects including and the *in vivo* effect on gastrointestinal tract of the selected plants/part of the plants were investigated as a part of pharmacological studies. The plant/part of the plant were subjected to systematic standardization/evaluation and along with the estimation of certain phytoconstituents like phenol as a part of phytochemical investigation.

The pharmacognostic evaluation of the leaves of *A.spinosa*, whole plants of *A. caudatus* and *A. viridis*, revealed encouraging results, which were within the prescribed limits. All though the ash values were within the required limit, *A. spinosus* and *A. caudatus* showed slightly higher total ash when compared to *A. viridis*, while, both acid insoluble and water-soluble ashes were slightly higher in *A. spinosus* as compared to *A. caudatus* and *A. viridis*. Microscopic evaluation showed glandular trichomes in *A.spinosa* while *A. caudatus* and *A. viridis* found to contain covering trichomes, as a differentiating character while other anatomical features appeared to be same.

*A. spinosus, A. caudatus* and *A. viridis* were dried and reduced to coarse powder and extracted successively by using soxhlet extraction method with petroleum ether, chloroform, methanol and water. The different extracts were
subjected to phytochemical analysis. Phytoconstituents like glycosides, saponins, alkaloids, steroids, proteins, amino acids, phenolic compounds, tannins and carbohydrates showed positive tests in the extracts.

The dried plant material of each of the selected drug was extracted separately with methanol by soxhlet extraction method for further studies.

HPTLC fingerprint of Methanol extracts of amaranthus showed the presence of flavonoids. MeAc and MeAs showed better antioxidant property than MeAv according to the HPTLC report. Rutin and quercetin content in the methanol extracts of each plant were estimated by HPLC. All the three plants showed almost same percentage of rutin and quercetin.

The total phenolic content on the other hand was found to be higher in methanol extract of *A. caudatus* compared to methanol extract of *A.spinosus* and *A. viridis*.

Methanol extract of *A. caudatus* showed significant antioxidant activity in DPPH radical scavenging, Nitric oxide scavenging, Superoxide anion scavenging, ABTS scavenging tests than methanol extract of *A. spinosus* and *A. viridis*. However, Methanol extract of *A. spinosus* showed potent hydroxyl scavenging activity compared to Methanol extract of *A. caudatus* and *A. viridis*.

Methanol extract of *A.spinosus*, *A. caudatus* and *A.viridis* were tested in alpha amylase inhibition assay by CNPG3 method. The results revealed that methanol extract of *A. caudatus* was potent alpha amylase inhibitor with IC$_{50}$ value 19.233 μg/ml than methanol extract of *A. spinosus* (77.806 μg/ml) and *A. viridis* (88.004 μg/ml) respectively. Acarbose used as standard.
The effect of methanol extract of *A. caudatus*, *A. spinosus* and *A. viridis* on hemoglycosylation was demonstrated by the degree of glycosylation of hemoglobin. MeAs showed potent inhibition of hemoglycosylation (34.63 % and 57.46%) than *A. caudatus* (36.19% and 56.07%) and *A. viridis* (23.43 % and 54.32%) at 0.5 and 1 mg/ml concentration. α-Tocopherol used as standard.

Analgesic potential of methanol extract of *A. caudatus*, *A. spinosus* and *A. viridis* were evaluated for peripheral pharmacological actions using acetic acid-induced writhing test. Methanol extracts of three plants were significantly (*P*<0.01) reduced writhing and stretching induced by 0.6 % acetic acid at dose of 10 ml/kg which was comparable to that of diclofenac sodium. In centrally acting analgesic property using hotplate and tail immersion method, at 200 and 400 mg/kg of methanol extracts showed significant analgesic activity (*P*<0.01) until the end of the experiment, which was comparable to that Morphine 5 mg/kg. The analgesic activity, decreased in the order of *A. spinosus* > *A. caudatus* > *A. viridis*.

In our study a significant (*P*<0.01) antipyretic effect were observed in the methanol extract of all three plants by reducing yeast-induced elevated body temperature in rats and their effects were comparable with that of the standard antipyretic drug paracetamol.

Effect of methanol extracts of *A. viridis*, *A. caudatus* and *A. spinosus* on gastrointestinal tract was assessed by gastrointestinal motility test, spasmogenetic effect on rat ileum and laxative activity.
The methanolic extract of *A. spinosus*, *A. caudatus* and *A. viridis* significantly (*P*<0.01) increased propulsion of the charcoal meal through the gastrointestinal tract at 100 mg/kg dose. No significant effects were observed at 50 mg/kg dose of methanol extracts. Senna used as standard.

Methanol extracts of three plants potentiates the concentration response curve of acetylcholine on isolated rat ileum. *A. caudatus* showed maximum contraction response when compared to *A. viridis* and *A. spinosus*.

In this study, methanol extracts of *A. spinosus*, *A. caudatus* and *A. viridis* at dose of 100 mg/kg significantly increased (*P*<0.01) fecal output of rats when compared to control group.

The results revealed that dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death. Methanol extracts showed significant (*P*<0.01) anthelmintic activity. The earthworms were more sensitive to the methanol extracts of *A. spinosus*, *A. caudatus* and *A. viridis* as compared to the reference drug piperazine citrate.

The antimicrobial activity of methanol extracts of amaranthus were tested by paper disc diffusion method against five pathogenic bacteria *E.coli*, *S.aureus*, *B. subtilus*, *Streptococcus*, and *K. pneumonia*. The highest antimicrobial potential was exhibited by methanol extract of *A. caudatus* followed by *A. spinosus* and *A. viridis*.

In conclusion, these findings suggest that *A. spinosus*, *A. caudatus* and *A. viridis* possess antioxidant, alpha-amylase inhibition, analgesic, antipyretic,
laxative, anthelmintic and antimicrobial activities. These results provide evidence to support the ethnomedicinal claims.

**Key words:** *Amaranthus spinosus, Amaranthus caudatus, Amaranthus viridis*, antioxidant activity, alpha amylase inhibition activity, analgesic activity, antipyretic activity anthelmintic activity, antimicrobial activity.