



SUMMARY

Malvaceae is a cosmopolitan family and *Abutilon* is one of the important genera of this family. Phytochemical investigations of two species of this genus, namely, *Abutilon indicum* L. and *Abutilon muticum* Del ex Dc. were carried out with respect to the isolation and characterization of their chemical constituents, seed oil fatty acids composition, antibacterial, antifungal, antioxidant and hepatoprotective activities of the crude extracts of different parts. The isolated compounds from the present work, were characterized by using various modern spectroscopic techniques.

The plant *Abutilon indicum* L. collected from the district, Lahore (Pakistan) showed the isolation and characterization of five new source and four known compounds. The compounds isolated and characterized for the first time were: 3,5,7-trihydroxy-4',6-dimethoxy-flavone [62], 3,5',5-trihydroxy-4'-methoxyflavone-7-0- β -D-gluco pyranoside [63], vasicine [64], lupeol [65] and methyl triacontanoate [66]. The isolated known compounds were; gallic acid [67] β -sitosterol [68], β -amyirin [69] and 4-hydroxy benzoic acid [70].

During these studies, seeds of *A. indicum* were subjected to proximate analysis. The results obtained were; moisture (5.9%), ash (2.3%), crude fiber (25.1%), crude protein (22.4%) and crude carbohydrate (22.92%). The seed ash was also analyzed for its mineral contents which were found to be (mg/100g); aluminium (4.02), cadmium (0.01), calcium (237.32), iron (3.13), lead (0.08), magnesium (176.50), phosphorus (212.15), potassium (261.01) and zinc (1.94). Lipids extracted from the finely ground seeds were fractionated into polar (3.7%) and non polar (95.2%) lipids. The fractionated lipids were analyzed into different lipid classes and were found to be; hydrocarbons (1.4%), sterol esters (8.3%), triglycerides (70.3%), free fatty acids (2.8%), diglycerides (3.2%), sterols (2.7%) and monoglycerides (6.5%). Total lipids and lipid fractions were analyzed for their fatty acids composition by gas chromatography. Triglyceride was found to be the major fraction (70.3 %) among neutral lipids. The lipids of *Abutilon indicum* were rich in unsaturated fatty acids and their composition was in general comparable to the fatty acid composition of the family Malvaceae. Oleic acid and Linoleic acids were found to be the predominant fatty acids in most of the lipids.

The antimicrobial activities of the crude extracts of different plant parts (leaves, stems, roots and seeds) of *A. indicum* were checked against three Gram-



negative bacteria, four Gram-positive rods and three fungi, using agar diffusion method. Leaf extract had shown enhanced activity against Gram-negative bacteria, *Salmonella typhimorium* (MIC; 339 μ g/ml) and Gram-positive bacteria, *Bacillus licheniformis* (MIC; 160 μ g/ml) in comparison to positive control. Root extracts showed low MIC value (239 μ g/ml) against *Escherichia coli*, which can be attributed to the presence of β -hydroxyl groups in its compounds. The antifungal activity of the leaf and stem extracts was negligible but root extract showed good activity (MIC; 640 μ g/l) against *Aspergillus niger*. Best activity (MIC; 235 μ g/l) was observed by root extract against *Trichoderma viride*. Antimicrobial activities of the seed oil were also checked and found to be higher than crude extracts. However *Salmonella typhimorium* was resistant to *A. indicum* seed oil. All the extracts tested had exhibited excellent activity compared to some of the typical antibiotics against most of the bacteria, showing their broad spectrum activity.

Extracts in organic solvents from aerial parts, roots and seeds were evaluated for their total antioxidant capacity (TAC), total phenolic content and total flavonoid content. Trolox equivalent antioxidant capacity (TEAC) of all the fractions of different plant parts was found employing ABTS and FRAP assays. The butanol extract of roots and chloroform extract of leaves of *A. indicum* showed highest TEAC (10.5 and 5.95 μ mol/g, respectively). The FRAP assay showed that antioxidant activity increased proportionate to the polarity of the solvent used for extraction. EC₅₀ and T_{EC50} values for the extracts were determined using DPPH free radical assay. Ethyl acetate extract showed EC₅₀ (68.3 μ g) at T_{EC50} (10 min), which indicated the presence of potent free radical scavengers in this plant species. The reaction kinetics with DPPH free radical indicated the presence of both the slow reacting and fast reacting antioxidant components in the extracts. The antioxidant/radical scavenging capacity of the extracts was found to be a dose-dependent activity. The butanol fraction of leaf extract of *A. indicum* gave comparable value to that of standard antioxidant; Trolox, by linoleic acid peroxidation method. The results obtained in the present study indicated *A. indicum* as the potential sources of natural antioxidants.

Abutilon muticum Del ex Dc. also belongs to the genus *Abutilon* of the family Malvaceae. Literature survey revealed that very little work had done on its seeds and no phytochemical work had so far been carried out on other parts of this plant species. This prompted us to carry out investigations on this plant source. Phytochemical



studies on *A. muticum*, collected from the district, Bahawalpur (Pakistan), had resulted in the isolation of eight new source compounds, which had never been isolated so far from this investigated source. The compounds isolated for the first time from *A. muticum* were: 3,4',5,6,7-pentahydroxy flavone [71], 3, 3', 4', 5, 7-pentahydroxy flavone-8-O- β -D-glucopyranoside [72], 3, 3', 4', 5, 7-pentahydroxy flavone [73], stigmasterol [74], benzoic acid [75], 1-tricosanol [76], cholesterol [77] and triacontyl palmitate [78].

Proximate composition of *A. muticum* seeds revealed that moisture content of this plant (6.3%) is quite close to *Brassica* and *Linseed* of the family Malvaceae. The protein content was appreciable (23.5 %) and similar to that of cotton (28.72 %) so *Abutilon* seeds could be recommended as protein supplements. As far as human nutritional aspects are concerned *A. muticum* seeds had significant mineral contents. Calcium was most abundant mineral (320.00) followed by potassium (312.32), phosphorus (235.02), magnesium (183.23), Iron (4.97) and aluminium (4.13) mg/100g, respectively. Lipids were also extracted and purified from the finely ground seeds. Seeds were rich in neutral lipids (94.7 %), while polar lipids represented only 3.6% of total lipids. The non polar lipids were analyzed into different lipid classes and were found to be; hydrocarbons (2.1%), sterol esters (7.4%), triglycerides (68.9%), free fatty acids (3.4%), diglycerides (2.7%), sterols (3.8%) and monoglycerides (6.4%). The polar lipids were fractionated into phospholipids (1.7%) and glycolipids (1.9%). Lipid fractions were further analyzed for their fatty acids composition. Oleic acid was found as the predominant fraction in neutral as well as polar lipids. Unsaturated fatty acids were higher, as compared to saturated fatty acid in all the lipid classes, which is the characteristic of vegetable oils. Pakistan being agricultural country undoubtedly had the capacity for the large scale production of *Abutilon* species. These results may therefore, offer a scientific basis for use of the seeds, both in human diet and other commercial products.

A. muticum extracts had shown greater antibacterial activity than *A. indicum* extracts. *A. muticum* root extracts were inactive against Gram-negative bacteria; *Proteus mirabilis*, but *Escherichia coli* and *Salmonella typhimorium* had shown encouraging results (MIC; 359 and 437 μ g/ml respectively). Leaf extracts were most active against *Bacillus licheniformis* (MIC; 230 μ g/ml) and root extract had shown good results against *Micrococcus luteus* (MIC; 264 μ g/ml). *Salmonella typhimorium*



was most resistant and *Bacillus licheniformis* was least resistant to *A. muticum* seed oil. Root extract showed good antifungal activity against *Aspergillus niger* (MIC; 196µg/ml) and stem extract against *Trichoderma viride* (MIC; 205µg/ml). The results obtained with both the *Abutilon* species may be considered very promising, indicating the potential for obtaining new antimicrobial agents.

Antioxidant activity of the crude extracts of different parts of *A. muticum* was checked. A wide range of poly phenolic content (0.448- 26.910 mg/g dry weight) and flavonoids content (0.111- 16.981 mg/g dry weight) was observed for different solvent fractions. TEAC values were measured by ABTS and FRAP assays. Butanol fraction of the root extract and ethyl acetate fraction of aerial parts showed highest TEAC values (14.208 and 13.21µmol/g respectively). The lowest TEAC values were obtained for n-hexane fraction of aerial parts (2.247µmol/g). The ethyl acetate and n-hexane fractions of different parts showed significantly stronger DPPH scavenging activity. Ethyl acetate fraction of aerial parts and roots gave EC₅₀ (102 and 96.3µg) at T_{EC50} (3 and 5 min), respectively. Root extracts had shown results comparable to the standard by linoleic acid peroxidation method. Taken collectively, these results lead to the conclusion that aerial part and root extracts of both *A. indicum* and *A. muticum* had powerful antiradical and antioxidant activity which may be helpful in controlling complications during degenerative diseases.

Hepatoprotective activity of aqueous methanolic extract of aerial parts of *A. muticum* was evaluated against paracetamol and CCl₄ induced hepatic damage in rabbits. The extracts at dose of 150 and 300mg/kg were administered orally. The substantially elevated enzyme levels were restored towards normalization significantly by the extracts. Silymarin was used as reference standard. The biochemical observations were supplemented with histopathological examination of rabbit liver sections. The results of this study strongly indicated that *A. muticum* had potent hepatoprotective action against paracetamol and CCl₄ induced hepatic damage in rabbits.

These analytical findings will provide a regional data base for these valuable herbs (*Abutilon indicum* L. and *Abutilon muticum* Del ex Dc.), which had not been explored so far. As *Abutilon* species grow wildly as weeds in tropical and sub tropical areas under harsh conditions, they may be produced on a large scale as value added products.