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RESEARCH ARTICLE

BIOASSAY OF MICROPROPAGATED *Andrographis paniculata*: AN OVERVIEW

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The present investigation is an overview on the biological assay done earlier on micropropagated plants of *Andrographis paniculata*. The effectiveness of the active compounds present in the plant extracts cause the production of growth inhibition zones that appear as clear areas surrounding the wells. Antimicrobial activity may be due to active components which are present in plant extracts. Regarding the solvent system for antioxidant assay, the method seems to work well with methanol or ethanol, neither of which seems to interfere with the reaction. The use of other solvents systems, such as almost neat extracts in water or chloroform, ethyl acetate, hexane and petroleum ether extracts, seems to give low values for the extent of reduction. The biochemical estimation results revealed the presence of carbohydrates, proteins, phenolics, flavonoids, saponins and alkaloids. This implies that the micropropagated plants are equally potent as that of the wild plants.

Key words: Biological assay, Antimicrobial activity, Antioxidant activity, Biochemical estimation, *Andrographis paniculata*

INTRODUCTION

Till date biological assay on several wild medicinal plants were done, while micropropagated plants are always avoided as they are subject to marked change in environment and are liable to be severely stressed until adequate precautions are taken. Leaves produced under high humidity/low transpiration potential, tend to have thinner cuticular wax layers and a more open mesophyll tissue. Under low light intensity, they may have reduced chlorophyll levels (Channarayappa, 2006). Antimicrobial activity on wild *Andrographis paniculata* are reported by Prajjal *et al*., 2003; Wanchaitanawong *et al*., 2005; Xu *et al*., 2006; Mishra *et al*., 2009 and Bobbarala *et al*., 2009 with aqueous, methanol, ethanol, hexane and chloroform extracts. Sheeja *et al*., 2006 and Rafat *et al*., 2010 evaluated the antioxidant potential of wild *Andrographis paniculata* whereas biochemical estimation with *in vitro* raised plants was mot made till date.

Kataky and Handique, 2010a evaluated the antioxidant potential with respect to inhibition concentration at 50% (IC_{50}) from micropropagated plants of *A. paniculata* with polar and non-polar solvents. Kataky and Handique, 2010b also evaluated the antimicrobial potential of the micropopagated plants of *A. paniculata* with common human pathogenic microbes. Biochemical estimation was also done on the micropropagated plants of *A. paniculata* (Kataky and Handique, 2010a,b) *Andrographis paniculata* is the only medicinal plant which has been used for bioassay experiments from the micropropagated plants. Thus, present investigation is an overview on biological assay done on the micropropagated 8 months old plants of *A. paniculata* reported by Kataky and Handique, 2010a,b.

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MATERIALS AND METHODS

Plant material

The field established micropropagated 8 months old plants of *A. paniculata* growing in the greenhouse of Department of Biotechnology, Gauhati University, Assam were used for the bioassay experiments.

Antimicrobial assay

Agar well diffusion method (Shanab *et al*., 2004; Kataky and Handique, 2010b) was used to determine the antimicrobial activity of various organic and aqueous extracts against gram negative *Klebsiella pneumoniae* (MTCC 432), *Escherichia coli* (MTCC 739), *Pseudomonas aeruginosa* (MTCC 443), gram positive *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 441) bacteria and the fungal pathogen *Candida albicans* (MTCC 227). MIC was done using two fold serial dilutions. For each bacterial and fungal strains control were maintained where pure solvents were used. Broad spectrum antibiotics viz. ampicillin, tetracycline and fluconazole at 1mg/ml concentrations were used as standards.

Antioxidant assay

The 2, 2- diphenyl-1, picrylhydrazyl radical (DPPH) method (Shyur et al. 2005; Kataky and Handique, 2010a) was used for determination of free radical-scavenging activity of the extracts. The radical scavenging activity is represented as % inhibition of DPPH radical and was

calculated according to the following formula- % inhibition = [OD control – OD sample / OD control] x 100 IC50 is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color) (Molyneux, 2004).

Biochemical estimation

The *A. paniculata* extracts were subjected to various biochemical tests to determine the total carbohydrate, total protein, total phenolics (Sadasivam and Manickam, 1991), total flavonoid (Pourmorad *et al*., 2006), total saponin (Obadoni and Ochuko, 2001; Edeoga *et al*., 2005) and total alkaloid, Harborne (1973) present in the crude extract.

RESULTS AND DISCUSSION

Antimicrobial assay

The antimicrobial activities of the plant extracted in different solvents varied greatly because there are many factors influence the active compounds present in the plant. The aqueous extract in the present study showed inhibitory effect on all the gram positive and gram negative bacteria (except *P. aeruginosa*). In almost all the test, the crude chloroform extract showed better inhibition against all the tested microbial strains. Ethyl acetate, acetone, DMSO extracts showed inhibitory effects against the gram positive bacteria but no inhibitory effect was observed with gram negative bacteria (Plate 1-6). It is noteworthy that gram positive bacteria *S. aureus* were inhibited more easily than were the gram positive ones. The chloroform extract of *A. paniculata* exhibited highest activity against *C. albicans* followed by the water extract. No significant inhibitory activity of the other solvent extract was observed against *C. albicans*. However, no inhibition was observed in control, which proves that solvents could not act as antimicrobial agents. The least MIC was found to be 15.625µg/ml (Kataky and Handique, 2010b).

The effectiveness of the active compounds present in the plant extracts cause the production of growth inhibition zones that appear as clear areas surrounding the wells. Antimicrobial activity may be due to active components which are present in plant extracts. However, some plant extracts (petroleum ether) were unable to inhibit antimicrobial activity against the tested microbial strains. These microbial strains may have some kind of resistance mechanisms for example: enzymatic inactivation, target site modification and decrease intracellular drug accumulation (Schwarz and Noble, 1999; Abeysinghe *et al*., 2006) or the concentration of the compound used may be not efficient (Abeysinghe *et al*., 2006).

Antioxidant study

In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (Koleva *et al*., 2002; Pourmorad *et al*., 2006). This assay is based on the measurement of the reducing ability of antioxidant towards DPPH. The ability can be evaluated by electron spin resonance (ESR) or by measuring the decrease of its absorbance. The widely used discoloration assay was first reported by Brand-Williams and co-workers, 1995. However, DPPH method was evidently introduced nearly 50 years ago by Marsden Blois, working in Standford University (Blois, 1958; Molyneux, 2004). Antioxidant assay are based on measurement of loss of DPPH color at 517nm after reacting with test compounds (Bondet *et al*., 1997) and monitored by a spectrophotometer (Prior *et al*., 2005).

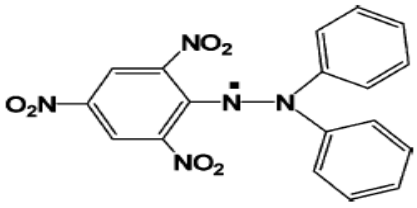

Figure 1: Structure of 2, 2- diphenyl-1, picrylhydrazyl (DPPH) (Prior *et al*., 2005).

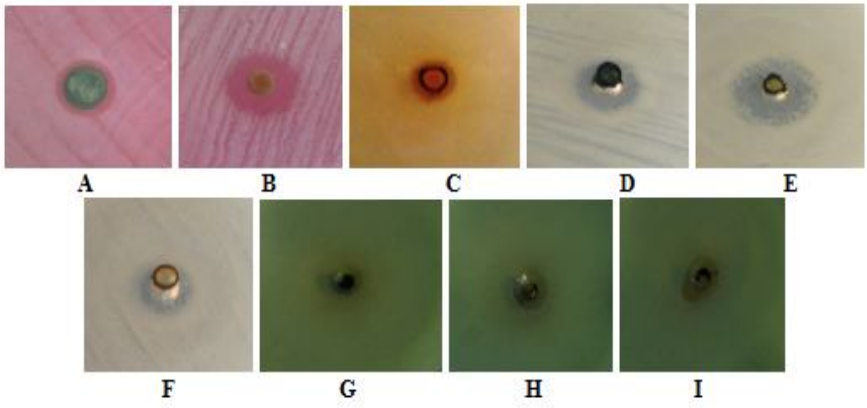

Plate 1: Chloroform extract of *A. paniculata* on gram negative bacteria

Klebsiella with **A.** Chloroform leaf extract; **B.** Chloroform stem extract; **C.** Chloroform root extract; *E.coli* with **D.** Chloroform leaf extract; **E.** Chloroform stem extract; **F.** Chloroform root extract; *P.aeruginosa* with **G.** Chloroform leaf extract; **H.** Chloroform stem extract; **I.** Chloroform root extract.

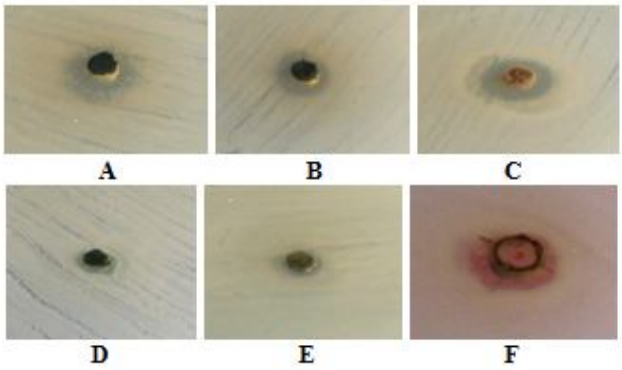

Plate 2: Aqueous extract of *A. paniculata* on gram negative bacteria
Klebsiella with **A.** Aqueous leaf extract; **B.** Aqueous stem extract; **C.** Aqueous root extract; *E.coli* with **D.** Aqueous leaf extract; **E.** Aqueous stem extract; **F.** Aqueous root extract

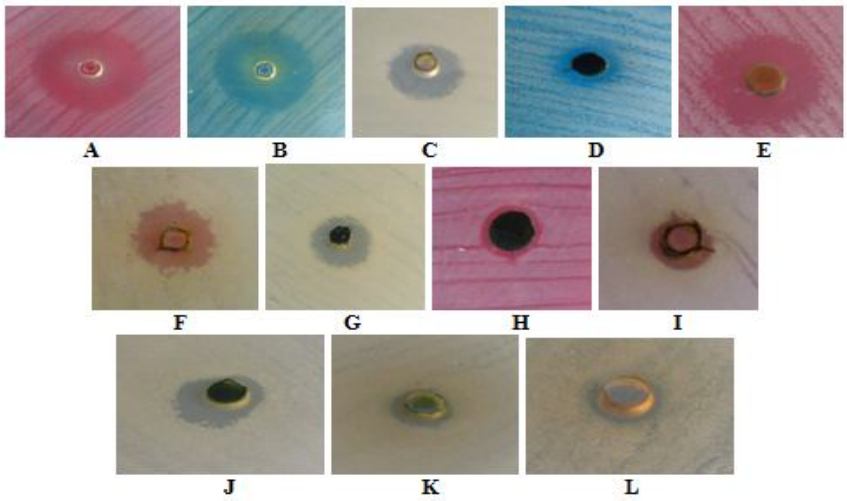

Plate 3: Chloroform and Ethyl acetate extract of *A. paniculata* on gram positive bacteria
Bacillus with **A.** Chloroform leaf extract; **B.** Chloroform stem extract; **C.** Chloroform root extract *Staphylococcus* with **D.** Chloroform leaf extract; **E.** Chloroform stem extract; **F.** Chloroform root extract; *Bacillus* with **G.** Ethyl acetate leaf extract; **H.** Ethyl acetate stem extract; **I.** Ethyl acetate root extract; *Staphylococcus* with **J.** Ethyl acetate leaf extract; **K.** Ethyl acetate stem extract; **L.** Ethyl acetate root extract

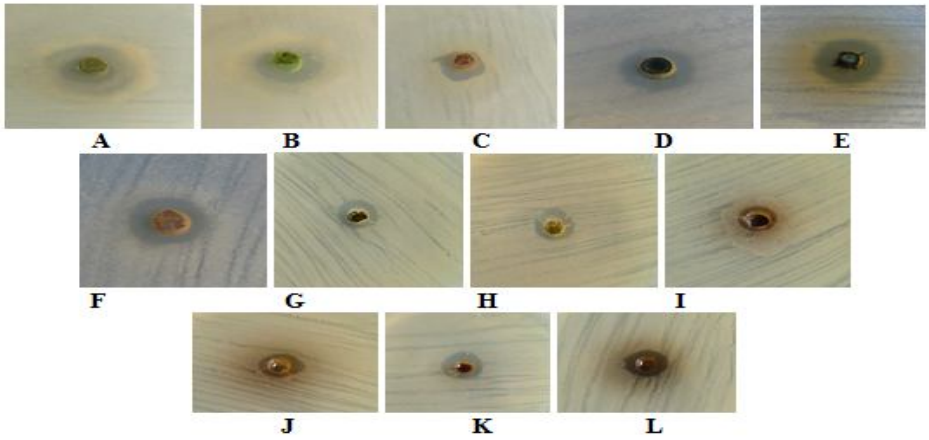

Plate 4: Acetone and Aqueous extract of *A. paniculata* on gram positive bacteria
Bacillus with **A.** Acetone leaf extract; **B.** Acetone stem extract; **C.** Acetone root extract; *Staphylococcus* with **D.** Acetone leaf extract; **E.** Acetone stem extract; **F.** Acetone root extract; *Bacillus* with **G.** Aqueous leaf extract; **H.** Aqueous stem extract; **I.** Aqueous root extract; *Staphylococcus* with **J.** Aqueous leaf extract; **K.** Aqueous stem extract; **L.** Aqueous root extract

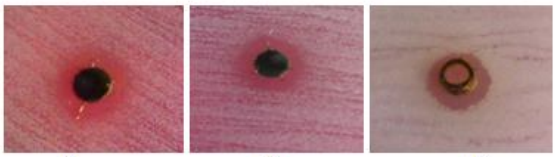

Plate 5: DMSO extract of *A. paniculata* on *Staphylococcus aureus*
Staphylococcus with **A.** DMSO leaf extract; **B.** DMSO stem extract; **C.** DMSO root extract

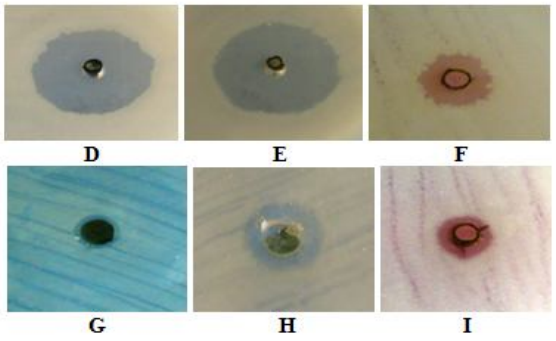

Plate 6: Chloroform and Aqueous extract of *A. paniculata* on *Candida albicans*
Candida with **D.** Chloroform leaf extract; **E.** Chloroform stem extract; **F.** Chloroform root extract;
Candida with **G.** Aqueous leaf extract; **H.** Aqueous stem extract; **I.** Aqueous root extract

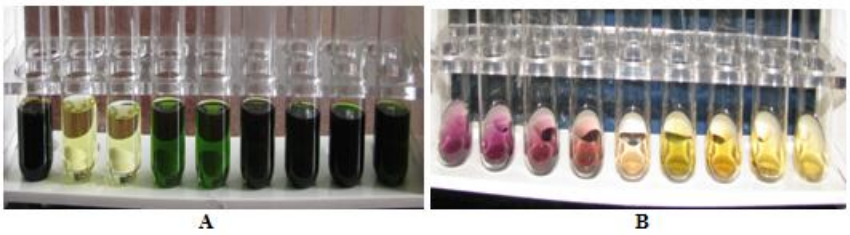

Plate 7: A. *Andrographis paniculata* extracts in various organic and aqueous extracts (Distilled water, Hexane, Petroleum ether, Ethyl acetate, Acetone, DMSO, Chloroform, Methanol and ethanol); **B.** Radical scavenging activity of various organic and aqueous extracts of *Andrographis paniculata* in Petroleum ether, Hexane, Ethyl acetate, Chloroform, DMSO, Acetone, Distilled water, Ethanol and Methanol

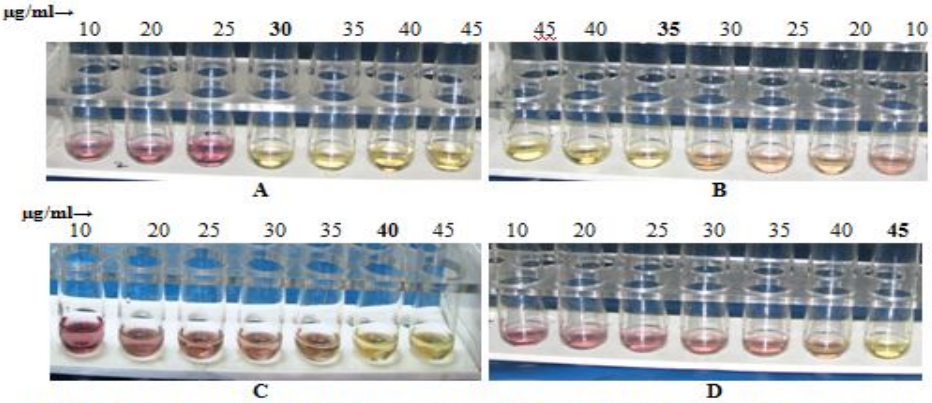

Plate 8: Radical scavenging activity of *A. paniculata* A. leaf/stem; B. leaves; C. stems; D. roots

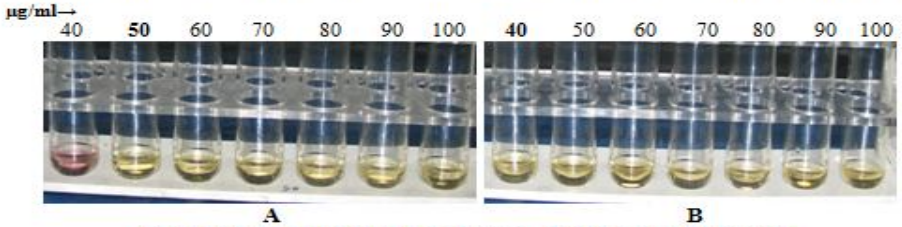

Plate 9: Radical scavenging activity of A. Ascorbic acid; B. Catechin

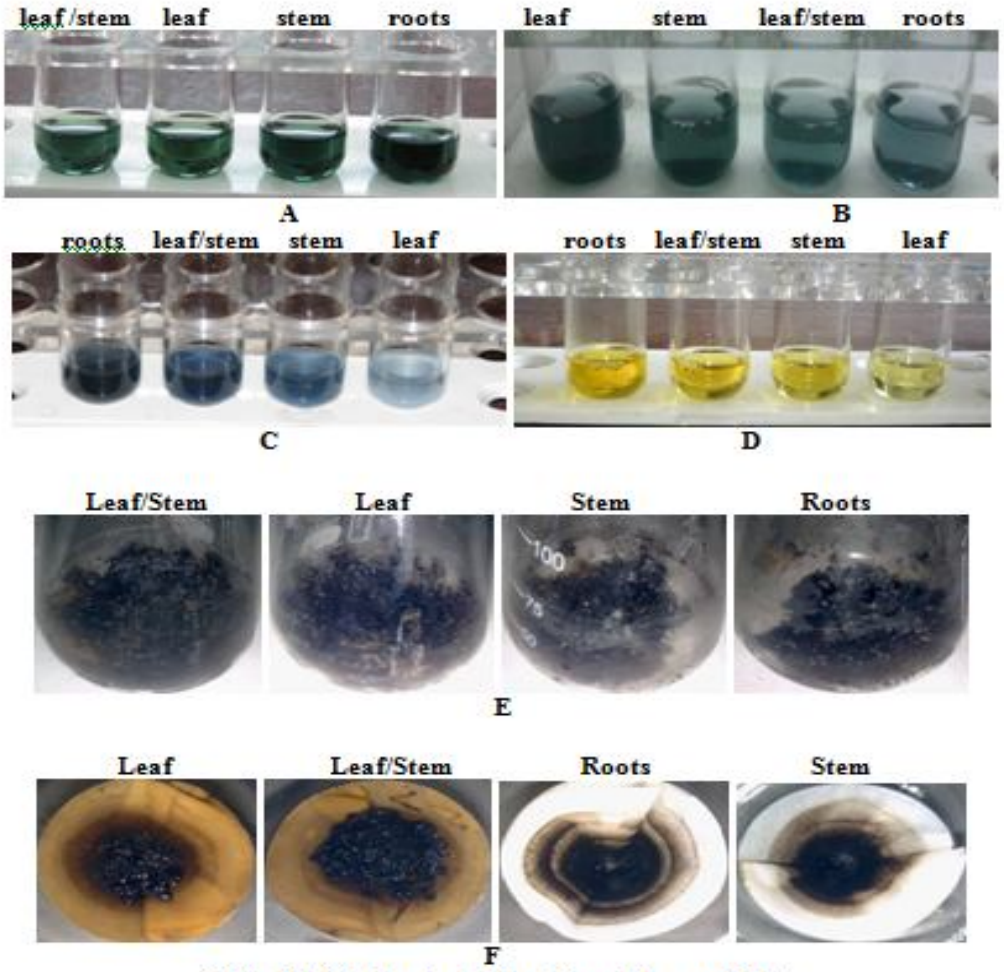

Plate 10: Biochemical estimation of *A. paniculata*

A. Total carbohydrate estimation; B. Total protein estimation; C. Total phenolics estimation; D. Total flavonoid estimation; E. Total saponin estimation; F. Total alkaloid estimation

Regarding the solvent to be used, the method seems to work well with methanol or ethanol, neither of which seems to interfere with the reaction. The use of other solvents systems, such as almost neat extracts in water or acetone, seems to give low values for the extent of reduction (Guo *et al*., 2001; Molyneux, 2004). This is in accordance with the present study. Methanol gives the maximum percentage inhibition followed by ethanol, distilled water, acetone and DMSO. Whereas, there is no reduction in the DPPH color in chloroform, ethyl acetate, hexane and petroleum ether extracts (Kataky and Handique, 2010a) (Plate 7). Methanol as a solvent used for DPPH radical scavenging activity has been reported by Pourmorad *et al*., 2006; Türkoğlu *et al*., 2007; Matkowsi *et al*., 2008 and Ayoola *et al*., 2008. The IC_{50} values for leaf/stem, leaves, stems and roots were found to be 30µg/ml, 35µg/ml, 40µg/ml and 45µg/ml respectively in methanolic extract (Plate 8) whereas, the IC_{50} values for ascorbic acid and catechin were 40µg/ml and 50µg/ml respectively (Kataky and Handique, 2010a) (Plate 9).

Biochemical estimation

Carbohydrates are the important components of storage and structural materials in plants. They exist as free sugars and polysaccharides.

Proteins are present in the living world, irrespective of the size of the organism, since they form the structural and functional basis of the cell (Sadasivam and Manickam, 1991). Polyphenols (tocopherols, phenolics, phenolic acids and flavonoids) from medicinal and aromatic plant possess a high antioxidant potential due to their hydroxyl groups and protect more efficiently against free radical related diseases such as atherosclerosis (Fuhrman and Aviram, 2001; Kris-Etherton *et al*., 2002; Vaya *et al*., 2003). Phenols, the aromatic compounds with hydroxyl groups are widespread in plant kingdom. They occur in all parts of the plant. Phenols are said to offer resistance to diseases and pest in plants (Sadasivam and Manickam, 1991). Plant phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers (Ayoola *et al*., 2008). Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants (Spencer, 2008). Flavonoids are widely distributed in plants fulfilling many functions. They also protect plants from attacks by microbes and insects. Flavonoids have been referred to as "nature's biological response modifiers" because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens.

They show anti-allergic, anti-inflammatory (Yamamoto and Gaynor, 2008), antimicrobial (Cushnie and Lamb, 2005), anti-neoplastic, anti-viral, anti-thrombic (Ayoola *et al*., 2008) and anti-cancer activity. Flavonoids also possess antioxidant activity, many of the biological functions, such as anti-mutagenicity, anti-canceroginicity and anti-aging, among others, originate from this property (Huang *et al*., 1992; Cook and Summan, 1996). Saponins on the other hand are of great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisones, diuretic steroids, vitamin D and cardiac glycosides (Evans, 2002; Adedapo *et al*., 2009). Saponins have many health benefits. Studies have illustrated the beneficial effects on blood cholesterol levels, cancer, bone health and stimulation of the immune system. The non-sugar part of saponins have also a direct antioxidant activity, which may results in other benefits such as reduced risk of cancer and heart diseases. In plants, saponins may serve as anti-feedants (Hartmut, 2006; Cornell University, 2008) and to protect the plant against microbes and fungi. Alkaloids usually have marked the physiological action on human or animals (Adedapo *et al*., 2009). Therefore, it is reseanoble to determine active constituent present in the plant extract.

The biochemical estimation results revealed the presence of carbohydrates, proteins, phenolics, flavonoids, saponins and alkaloids. Thus, the antimicrobial and antioxidant activity of *A. paniculata* is responsible for the phenolics, flavonoids, saponins and alkaloids. And carbohydrates and proteins give the nutritive value of *A. paniculata*. (Plate 10). On the basis of presented results, we can consider the *in vitro* raised plants of *Andrographis paniculata* are equally potent as that of the wild plants.

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