Despite the recent interest in molecular modeling, combinatorial chemistry, and other synthetic chemistry techniques by Pharmaceutical companies and funding organizations, natural products, particularly medicinal plants, remains an important source of new drugs, new drug leads, and new chemical entities. It is evident that, natural products have played a vital role in drug discovery, by contributing a wide variety of phytochemicals for the treatment of cancer, cardiovascular disease, infections related with viral and microbial origin and other health disorders.

It has been noted that there is a great probability of finding compounds with different biological activities from plants with reputed medicinal properties over plants collected at random. It gives an idea about the importance of natural products, especially plants in modern day drug discovery. This encouraged the researchers to investigate the traditional medicinal plants for their various biological properties and number of Indian traditional medicinal plants have been a source for a variety of new phytochemicals with diversified biological properties, Hence, the present study was taken up to investigate medicinal plants of genus Sida and compare the antiviral properties of different species of Sida against HSV I & II, Adenovirus type VIII, Poliovirus type I and Influenza virus type A PR/8 and IFV-296 strains.

The plant species of the genus Sida are well known for their use in traditional medicine. In the present investigation four plants of the genus Sida namely *Sida Cordifolia Linn.*, *Sida acuta Burm.*, *Sida retusa L.*, and *Sida spinosa Linn* were collected, authenticated and extracted in a soxhlet extractor with non polar to polar solvents (Pet. Ether, Toluene, Chloroform, Ethyl acetate, Acetone, Hydroalcohol and Methanol). A total of twenty five extracts were prepared from these four plants.

The phytochemical studies confirmed the presence of alkaloids, flavonoids, carbohydrates, proteins, phenols and phytosterols in different extracts.

*In vitro* cytotoxicity studies were carried out with MTT and SRB methods because *in vitro* cytotoxicity methods are important tools to enhance the understanding of hazardous effects caused by chemicals or bioactive components, which avoids usage of animals (Broadhead and Combes, 2001). These tests provide useful and necessary information in defining basal cytotoxicity, which is commonly used as a starting point in an integral assessment of
potential *in vivo* toxicity of chemicals or active components in foods. The endpoints frequently used in cytotoxicity testing are based on the breakdown of the cellular permeability barrier, reduced mitochondrial function, changes in cell morphology, and changes in cell replication (Eisenbrand *et al.*, 2002).

In the present investigation extracts and isolated fractions were screened to determine their cytotoxicity towards the four cell cultures (Vero, HEp-2, A-549 and MDCK) to decide the dose which should be non toxic to the cell culture used for antiviral studies. Among the extracts and fractions tested, the pet ether extract of *Sida cordifolia* showed higher cytotoxicity towards Vero, HEp-2, A-549 and MDCK cell cultures with CTC\(_{50}\) values 103µg/ml, 69.75µg/ml, 93.65µg/ml and 108.2µg/ml, respectively. The toluene extract of *Sida acuta* showed comparatively more toxicity to Vero, HEp-2, A-549 and MDCK cell cultures with CTC\(_{50}\) values 31µg/ml, 41.33µg/ml, 33.16µg/ml and 33.12µg/ml, respectively. The ethyl acetate extract of *Sida spinosa* showed higher cytotoxicity with CTC\(_{50}\) value 50.12µg/ml against MDCK cell culture. The moderate toxicity was seen with acetone and toluene extracts of *Sida retusa* with CTC\(_{50}\) values 105µg/ml and 110µg/ml and 120µg/ml and 110µg/ml against Vero and HEp-2 cell cultures. Among the isolated fractions tested the highest cytotoxicity was seen with FT, with CTC\(_{50}\) value of 64.5µg/ml against HEp-2 cell culture.

The *in vitro* antiviral activity was carried out by CPE inhibition assay, virucidal assay, MTT antiviral assay, time of addition studies, plaque reduction assay and immunofluorescence assay. The virus challenge dose of 10 TCID\(_{50}\) and 100 TCID\(_{50}\) were used against HSV-I & II, Adenovirus type VIII, Polio virus type I and Influenza virus type A (strain A/Puerto Rico/8/1934 H1N1) and IFV-296. The 100 PFU were used for HSV TK- and influenza virus type A (H1N1). The cytopathic inhibition assay is usually the first step in screening large number of compounds for their anti-viral activity. All the twenty five extracts were screened for antiviral activity by CPE inhibition assay and virucidal assay. Among all the extracts tested, the extracts of *Sida cordifolia* showed the promising protection from viral infection at highest virus challenge dose and at lowest concentration used, so those extracts were taken further for the advanced studies like MTT antiviral assay, mode of antiviral action, plaque reduction assay and Immunofluorescence assay.
At 100 TCID$_{50}$ of HSV-I challenge dose, Tol, HA and MeOH extracts of *Sida cordifolia* showed promising cell protection with IC$_{50}$ values 37µg/ml, 12µg/ml and 22µg/ml when challenged with 50µg/ml treated dose. Those values were compared with acyclovir, standard drug used for the study which showed 98.33% protection with IC$_{50}$= 7µg/ml. When antiviral activity was checked with isolated fractions and standard compounds against HSV-I, FHA showed 80.00% cell protection at 100µg/ml with IC$_{50}$= 74µg/ml, followed by FT, 82% at 50µg/ml with IC$_{50}$= 40µg/ml. The highest protection was seen with FMeOH, 66.66% at 25µg/ml with IC$_{50}$= 21µg/ml.

Against HSV-II, the HA extract of *Sida cordifolia* offered maximum cell protection at 50 µg/ml it showed 94.00% protection with IC$_{50}$= 19µg/ml followed by Pet. Ether at 50µg/ml offered 88.33% cell protection with IC$_{50}$= 19µg/ml. The isolated fraction FT at 50µg/ml protected cell by 56.00% with IC$_{50}$= 45µg/ml.

The antiviral activity of most effective three extracts i.e., Tol, HA, MeOH was confirmed by plaque reduction assay on HSV TK- viral strain. The HA extract showed highest selectivity index of 5.31 followed by MeOH (SI=5.29) and Tol extract (SI=3.94)

Against Adenovirus type VIII, the maximum cell protection was offered by HA extract of *Sida cordifolia* at 50µg/ml it showed 92.33% protection with IC$_{50}$= 18µg/ml followed by Tol at 100µg/ml with IC$_{50}$= 47µg/ml. The IC$_{50}$ of extracts were compared with standard drug ribavirin which showed 98.00% cell protection with IC$_{50}$= 9µg/ml. The isolated fractions did not show any activity against adenovirus type VIII.

Against Poliovirus type I, In CPE inhibition assay all the extracts of *Sida cordifolia* offered 100% protection microscopically which was confirmed with MTT antiviral assay where the highest cell protection was observed with Pe extract of *Sida cordifolia* at 50µg/ml it offered 90% protection with IC$_{50}$= 18µg/ml followed by HA and Tol extracts at 50 µg/ml showed 89.33% and 88.66% virus inhibition with IC$_{50}$= 19µg/ml. The results were compared with standard Guanidine hydrochloride which showed 99.00% protection at 12.5µg/ml with IC$_{50}$= 8µg/ml. all other extracts showed moderate activity. The FT and FHA showed protection with IC$_{50}$= 17µg/ml and 36µg/ml.

Against Influenza virus type A H1N1, the antiviral activity was performed with two strains of influenza A i.e., PR-8/34 and IFV-296. The maximum cell protection was offered by HA extract of *Sida cordifolia* against both the viral strains. The activity was confirmed by plaque reduction assay where hydroalcohol extract offered highest selectivity index of 41.
and 13.66 against IFV-296 and PR-8 strains. Further confirmation was done by immunofluorescence assay where HA extract at high treated dose (100µg/ml) suppress the expression of viral antigen in the infected cells. The FHA offered cell protection at 50µg/ml against both the influenza strains used. The results were compared with the standard drug oseltamivir which showed maximum virus inhibition at 10µg/ml it offered 94% protection with IC₅₀= 7µg/ml.

The in vivo antiviral activity was carried out on the three best extracts out of twenty five extracts. The Tol, HA and MeOH extracts of Sida cordifolia were selected for in vivo against HSV-I 7401 wild type virus. Two most effective models were used for the study. Among the extracts tested the toluene extract of Sida cordifolia showed the most potent activity in vivo which was comparable with the standard drug Acyclovir. Tol at 250 mg/kg significantly delayed the development of skin lesions and prolonged the mean survival time (P<0.05 by Chi-square test) as compared with control. The Tol extract significantly reduced the virus titer in skin and brain when compared with control. Further the Tol extract (4.43 ± 0.15 log₁₀ PFU/organ, P<0.0001) showed better reduction of virus titer in brain than the acyclovir (4.61 ± 0.12 log₁₀ PFU/organ, P<0.0001) the standard drug.

Sida cordifolia a plant belonging to Sida genus showed promising antiviral activity against DNA (HSV I & II, HSV TK- and Adenovirus type VIII) and RNA (Poliovirus type-I and Influenza virus type A (H1N1). The extracts Tol, HA and MeOH showed promising activity which contains alkaloids and phytosterols and those extracts were further fractioned to separate the alkaloids from HA and MeOH and phytosterols from Tol extracts. The activity of isolated fractions and the crude extracts were compared. Earlier studies by research groups working on Sida cordifolia have concluded that the plant contains ephedrine and pseudoephedrine, vasicinol and vasicinone and β-sitosterol and stigmasterol in its various parts (Franzotti, 2000). The quantities are low, with less than 2% of ephedrine and pseudoephedrine found in the leaves of Sida cordifolia. Ephedrine is known to stimulate the central nervous system (CNS), and such can enhance weight loss. Traditionally nutrition companies used plants such as Ma-Huang (Ephedra plant), because it contained relatively large amount of ephedrine, in their weight loss products. However, since this product was banned in many countries including the USA and UK, they are now looking for alternatives. Sida cordifolia, with its ephedrine and pseudoephedrine has
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gained a lot of interest and is now sold by many of these companies (Ghosal et al., 1975). Very recently, methanol extracts of *Sida cordifolia* showed potent antioxidant and anti-inflammatory activity (Swathy et al., 2010).

With the present *in vitro* and *in vivo* investigation it is concluded that the toluene extract of *sida cordifolia* L. is most potent followed by hydroalcohol and methanol extract when compared to the standard Acyclovir. However, further in depth clinical investigation may be required to confirm these studies.