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A Comparative Study on Anti-Microbial Activity Exhibited by *Stevia rebaudiana l. Bertoni in* Methanol and Chloroform Extract

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Abstract: ***Stevia an exotic medicinal plant, is the world's only natural sweetener with zero calories, zero carbohydrates and a zero glycaemic index. The leaves of stevia are the source of diterpene glycosides, stevioside and rebaudioside. Because of these components, this herb is a natural sweetener plant, estimated to be 300 times sweeter than sugar cane. Plant tissue culture is a suitable approach for micro propagation and production of valuable secondary metabolites of plants. A present procedure has been outlined for antimicrobial screening of a medicinal herb, Stevia rebaudiana Bertoni. In vivo grown leaf extracts in different solvent system were screened for potential antimicrobial activity with zone of inhibition against standard and medically important bacterial and fungal strains by disc diffusion method. The chloroform and methanol extract exhibited a concentration dependent antibacterial and antifungal inhibition. The methanolic extracts of in vivo plants of Stevia showed best antibacterial and antifungal activity against a number of microorganisms. The resultant of which proved the Stevia's potential antimicrobial agent as non-antibiotics sources. Therefore, commercial manufacture of active constituents from these improved elite lines would be useful and profitable.***

Keywords: *Stevia rebaudiana*, Regeneration, Antimicrobial Activity, Leaf Extract, Disc diffusion method

1. Introduction

Medicinal plants are the most important source of life saving drugs for majority of world population. In Indian subcontinent, plant oriented drugs have been used extensively from a very long time. Some plant based biological compounds isolated from herbs hence been explored for the growth inhibition of pathogenic microbes due to their antimicrobial potential. *S. rebaudiana* (Bertoni) is an herbaceous perennial plant of the *Asteraceae* family. It has an alternate leaf arrangement and herbaceous growth habit (Singh and Rao, 2005). *Stevia* plant has many sterols and antioxidant compounds like *triterpenes, flavonoids*, and *tannins*. Some of flavonoid polyphenolic anti-oxidant phyto-chemicals present in *Stevia* is *kaempferol, quercetin, chlorogenic acid, caffeic acid, isoquercitrin, isosteviol*…etc. Studies found that kaempferol can reduce risk of pancreatic cancer by 23% (American journal of epidemiology).*Stevia* is also proved to inhibit the growth of certain bacteria and other infectious organisms hence used against wounds sores and gum disease. It may also explain while the herb is advocated for anyone who is susceptible to yeast infections or reoccurring streptococcal infections, two conditions that seem to be aggravated by white sugar consumption. Some people also suggested that *Stevia* is very good working on cold and flu. The biological activity for compound *Stevia* has been studied by Tomita *et al.* (1997). They have studied bactericidal activity of a fermented hot water extract from *S. rebaudiana* Bertoni towards enterohemorrhagic *Escherichia coli* and other foodborne pathogenic bacteria. Other microorganism like *Salmonella typhimurium, Bacillus subtilis*, and *Staphylococcus aureus* has been found to be inhibited by fermented leaf extract. Similar antimicrobial studies of leaf extract of *Ocimum gratissimum* on selected diarrhea causing bacteria in South-Western Nigeria has also been studied. The medicinal properties are attributed to the primary and secondary metabolites synthesized by the plants. Antibacterial activity of various plant extracts has been studied by many workers. The effects of plant extracts on bacteria have been studied by a very large number of researches in different parts of the world. Much work has been done on ethanolic extracts plants in India.

2. Antimicrobial Activity

The crude leaf extract of *S. rebaudiana (in vivo)* in different system were tested against different pathogens. *Stevia* proved to be very effective antimicrobial agent. Result showed the positive response in methanolic extract Table 9 (a and b), was the most effective against all the bacteria. The higher antibacterial activity of the methanol and chloroform extracts may be due to the greater solubility of the extract in these organic solvents. Maximum number of bacterial strains was found susceptible to Stevia extracts. Antibacterial property of *Stevia rebaudiana* extracts in various solvents on four bacteria viz. E. coli, B. subtilis, S. mutans and S. aureus and six different fungal strains were. However, only few fungi found inhibited by leaf extracts by Debnath, 2008.

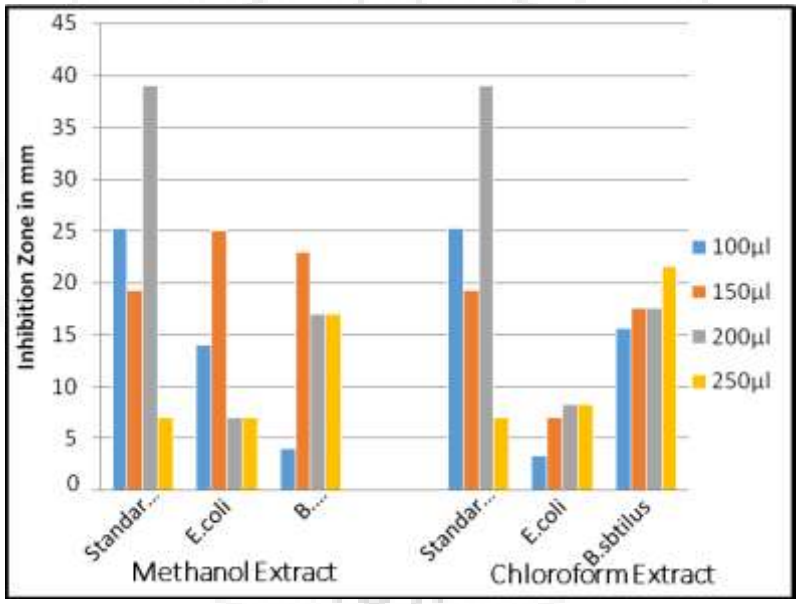
The medicinal properties were attributed to the primary and secondary metabolites synthesized by the plants (Faizi *et al.*, 2003). In our studies we found that all the bacteria were inhibited by the *S. rebaudiana* extract in the various solvent although only a one fungi showed inhibition to the leaf extract. Adebolu and Oladimeji (2005) also did similar test and found out the antimicrobial activity of the leaves of *Ocimum sp.* Parekh *et al.*, (2005) worked on efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity in twelve medicinal plants. Tomita *et al.*, (1997) reported bactericidal activities of fermented hot water extract from *S.rebaudiana*, towards enterohemorrhagic *E. coli* O157:47 and other food borne pathogenic bacteria. Methanolic extract was found to be the best solvent to result in good antimicrobial activity.

It may be made possible that the secondary metabolite "stevioside" was responsible for the antimicrobial activity (Nakamura and Tamura, 1985). It may also be concluded that the secondary metabolite was most soluble and acted as antimicrobial substance when it was in methanolic solvent system. On dilution of the plant leaf extract (for determining of the minimum inhibitory concentration), better susceptibility and zone of inhibition was observed in many cases. It may be due to the pure extract which was more viscous and was unable to permeate and diffuse properly in the medium, but after dilution it could easily diffused in to the medium (Parekh *et al.*, 2005). The more antimicrobial property of the *in vitro* regenerated plantlets may be due to more secondary metabolite and thus these plantlets used be a source of elite plantlets. Hence this plant can be further subjected to isolation of the therapeutic antimicrobials and further pharmacological evaluation.

The *in vivo* antimicrobial activity of methanol and chloroform extracts of dried Stevia leaves (*in vivo*) were shown in Table 9, a and b.

Table 9(a): Antibacterial activity of methanol and chloroform extracts of Stevia *rebaudiana* leaves *in vivo* (zone of inhibition in mm)

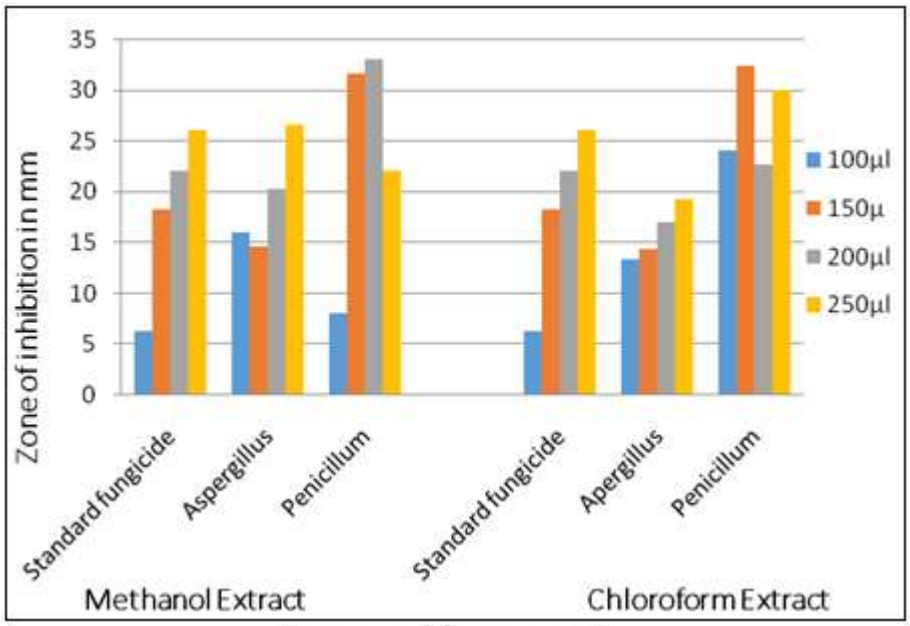
S/No.	Solvent system	Conc. (%)	Inhibition zone in mm		
			E.coli	B. subtilis	Standard antibiotic
1.	Methanol Extract	100µl	14	4	25.3
2.		150µl	25	23	19.3
3.		200µl	7	17	39
4.		250µl	7	17	7
5.	Chloroform extract	100µl	3.3	15.6	25.3
6.		150µl	7	17.6	19.3
7.		200µl	8.3	17.6	39
8.		250µl	8.3	21.6	7



Graph 4: Effect ofantibacterial activity of methanol and chloroform extracts of *Stevia rebaudiana* leaves *in vivo* (zone of inhibition in mm)

Table 9(b): Antifungal activity of methanol and chloroform extracts of Stevia *rebaudiana* leaves in vivo (zone of inhibition in mm)

S/No.	Solvent system	Conc. (%)	Inhibition zone in mm		
			Aspergillus	Penicillium	Standard fungicide
1.	Methanol Extract	100µl	16	8	6.3
2.		150µl	14.6	33.1	18.3
3.		200µl	20.3	33	22
4.		250µl	26.6	33.6	26
5.	Chloroform extract	100µl	13.3	24	6.3
6.		150µl	14.29	28.3	18.3
7.		200µl	17	22.6	22
8.		250µl	19.3	30	26



Graph 4: Effect ofantifungal activity of methanol and chloroform extracts of Stevia *rebaudiana* leaves *in vivo* (zone of inhibition in mm)

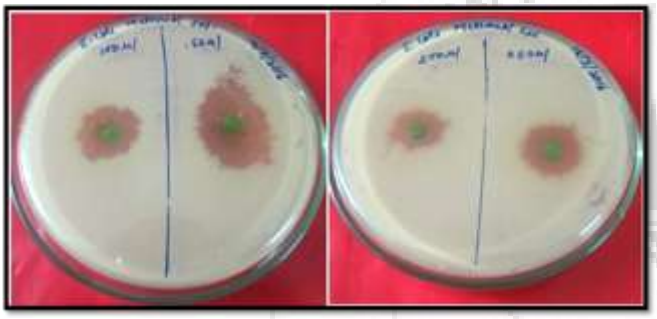


Figure 9(A-B): Antibacterial activity (E. coli) against methanol extract at different concentration
A. Effect of antibacterial (E.coli) activity against methanol extract (100µl and 150µl)
B. Effect of antibacterial (E.coli) activity against methanol extract (200µl and 250µl)

Figure 10(A-B): Antibacterial activity (E. coli) against chloroform extract at different concentration
A. Effect of antibacterial (E.coli) activity against chloroform extract (100µl and 150µl)
B. Effect of antibacterial (E.coli) activity against chloroform extract (200µl and 250µl)

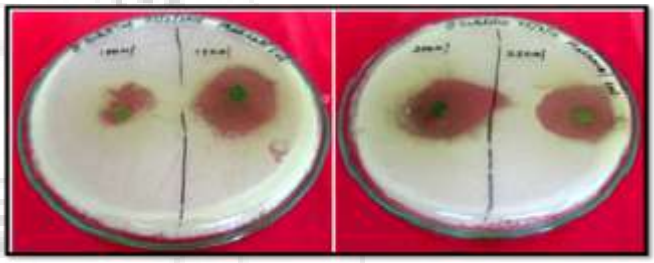


Figure 11 (A-B): Antibacterial activity (B. subtilus) against methanol extract at different concentration
A. Effect of antibacterial (B. subtilus) activity against methanol extract (100µl and 150µl)
B. Effect of antibacterial (B. subtilus) activity against methanol extract (200µl and 250µl)

Figure 12 (A-B): Antibacterial activity (B. subtilus) against Chloroform extract at different concentration
A. Effect of antibacterial (B. subtilus) activity against chloroform extract (100µl and 150µl)
B. Effect of antibacterial (B. subtilus) activity against chloroform extract (200µl and 250µl)

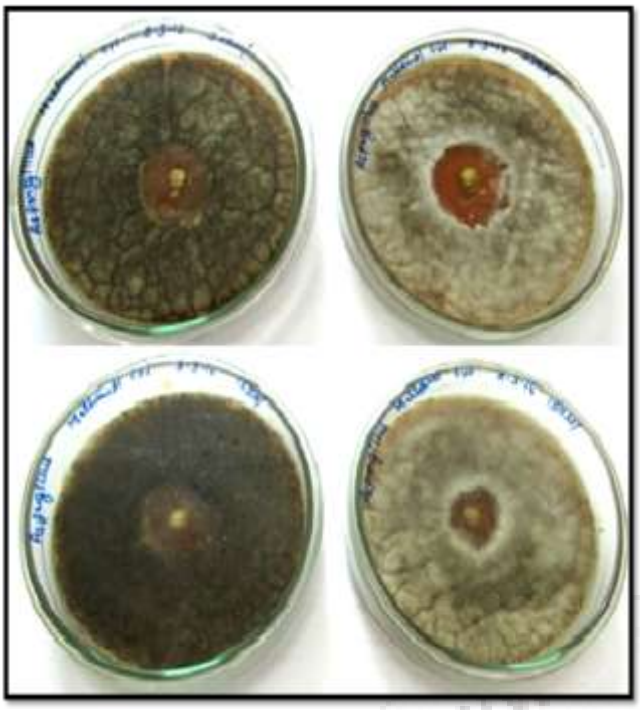


Figure 13 (A-D): Antifungal activity (Aspergillus) against methanol extract at different concentration

A. Effect of antifungal (Aspergillus) activity against methanol extract (100µl)

B. Effect of antifungal (Aspergillus) activity against methanol extract (150µl)

C. Effect of antifungal (Aspergillus) activity against methanol extract (200µl)

B. Effect of antifungal (Aspergillus) activity against methanol extract (250µl)

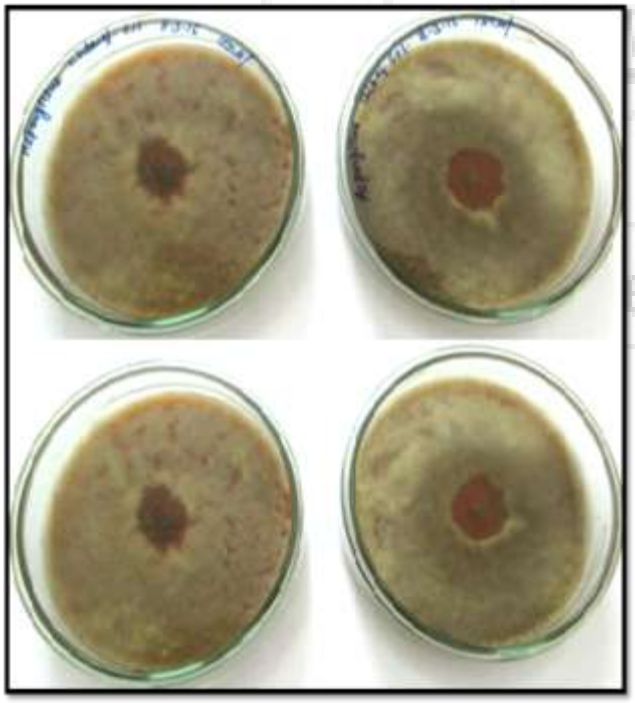


Figure 14 (A-D): Antifungal activity (Aspergillus) against chloroform extract at different concentration

A. Effect of antifungal (Aspergillus) activity against chloroform extract (100µl)

B. Effect of antifungal (Aspergillus) activity against chloroform extract (150µl)

C. Effect of antifungal (Aspergillus) activity against chloroform extract (200µl)

B. Effect of antifungal (Aspergillus) activity against chloroform extract (250µl)

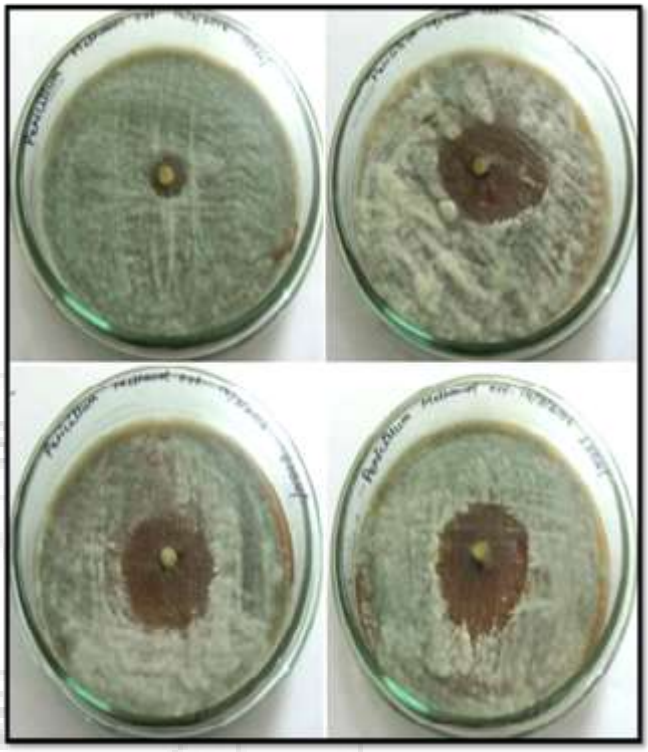


Figure 15 (A-D): Antifungal activity (Penicillium) against methanol extract at different concentration

A. Effect of antifungal (Penicillium) activity against methanol extract (100µl)

B. Effect of antifungal (Penicillium) activity against methanol extract (150µl)

C. Effect of antifungal (Penicillium) activity against methanol extract (200µl)

B. Effect of antifungal (Penicillium) activity against methanol extract (250µl)

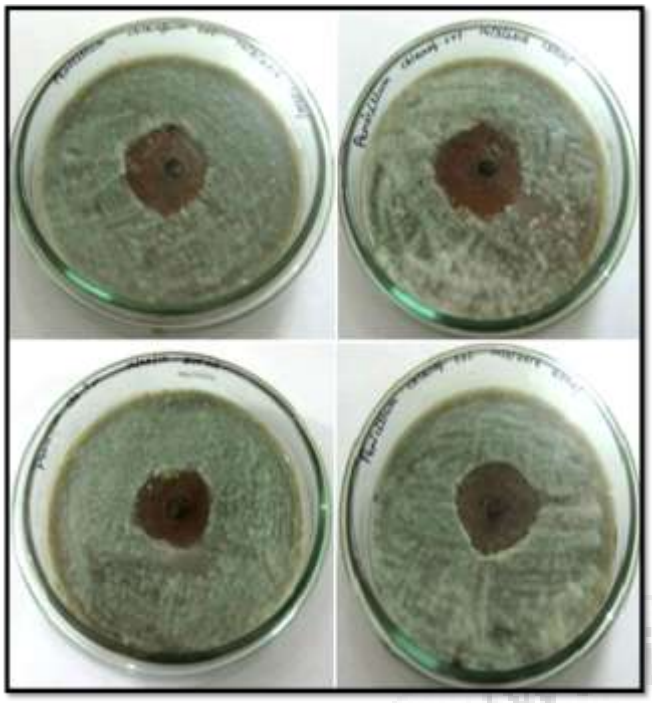


Figure 16 (A-D): Antifungal activity (Penicillium) of the *Stevia rebaudiana in vivo* plants leaves extracts

A. Effect of antifungal (Penicillium) activity in chloroform extract (100µl)
B. Effect of antifungal (Penicillium) activity in chloroform extract (150µl)
C. Effect of antifungal (Penicillium) activity in chloroform extract (200µl)
B. Effect of antifungal (Penicillium) activity in chloroform extract (250µl)

3. Conclusion

In vivo grown leaf extracts in different solvent system were screened for potential antimicrobial activity against medically important bacterial and fungal strains by disc diffusion method was performed to check the susceptibility of *Stevia* against certain pathogens. Using Disc diffusion method, the compounds present in methanolic extract has shown high microbial activity. Although all the individual extracts (obtained from cultivated *Stevia* plant) show potential antimicrobial activity as compared to standard ampicilin and fungicide but the activities were lesser than standard.*Stevia* proved to be very effective antimicrobial agent. The higher antibacterial activity of the methanol and chloroform extracts may be due to the greater solubility of the extract in these organic solvents.

4. Future Aspects

Antimicrobial activities of various herbs and spices in plant leaves, flowers, stems, roots, or fruits have been reported by many workers. *Stevia* has the ability to inhibit the growth of certain bacteria, helps to explain its traditional use in treating wounds, sores and gum disease. *Stevia* plant could be proved as future potential antimicrobial agent as non-antibiotics sources. Leaf extracts of *Stevia* may be an ideal candidate for further research into their uses for food preservation as well aspharmaceutical and natural plant-based products. The study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.The present investigation endow with the basic information about new non antibiotic drug molecules of plant origin, especially methanol extract if *Stevia* leaves which is found to be potent enough in exhibiting substantial antimicrobial activity against pathogens. Extraordinary anti-fungal activity higher than the standard fungicide used against plant pathogens may be substituted as potent bio-fungicide. Therefore, these molecules could be proved as future potential candidate either as non-antibiotic pharmaceuticals or food preservatives and plant micro-biocides after proper toxicity study in plant and animal models and clinical trials are addressed.

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