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Antimicrobial comparative competence of phytochemical extract from Calendula officinalis and Citrus sinensis

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ABSTRACT
The selected plant materials are known such as COMF (Calendula officinalis: Marigold flowers) and CSOFP (Citrus sinensis: Orange fruits peel) have good antimicrobial efficiency. Selected organic solvents such as chloroform (Chl.), ethanol (Eth.), methanol (Met.) and water (Wat) were selected for extraction of phytochemicals. The different kinds of phytochemicals have been used for each in the soxhlet extraction. In which the total quantity of 3.180 g from 100 g of COMF and total 3.090 g from 100 g of CSOFP samples has been used for phytochemicals extraction. The growth inhibition of microbial on NAM by disc diffusion methods using and MIC (minimum inhibitor concentration) has been compared with standard medicine.

Keywords: COMF, CSOFP, MIC, NAM, Phytochemical, Antimicrobial.

I. INTRODUCTION:

The use of natural resources such as phytochemicals derived from a variety of plant products and their useful parts is known to break the spread rate of diseases caused by microorganisms in primary treatment and as antimicrobial therapeutic agents.[1.2] Plant extracts of marigold flowers and orange peel have many medicinal benefits as well as have good antimicrobial properties. Plant extract ingredients contain a variety of phytochemicals including carotenoids, chlorophyll, saponins, flavonoids and triterpenes, etc., which are easily extracted in various solvents such as chloroform, ethanol, methanol and water, etc.[3,4] The selection of organic solvents the successful determination of biologically active compounds from plant material basically depends on the type of solvent used in the extraction process.[5,6] The properties of a good solvent in plant extraction include low toxicity, ease of evaporation at low heat, promoting rapid physiological absorption of the extract, to prepared by soxhlet extractor.[7-9] The marigold flower and orange peel are responsible for this antimicrobial action against both bacteria and fungi and against air-borne microorganisms, in addition to significantly reducing the problem of environmental pollution.[10,11] In ancient times, rubbing extracts of marigold flowers and orange peel on the skin prevented inflammation. The skin affected by wasp or bee stings helps to reduce pain, swelling and inflammation and also prevents microbial infection which is mainly used externally to treat bruises, wounds, eczema, skin disorders, hemorrhoids and burns. This research has been carried out in vitro as applied to the culture of microbial at various concentrations of extract of phytochemical substrates to determine the inhibition of microbial growth and found on the efficiency against microbial of the above extracted substrate.[12,13]They can be used efficiently as drugs as the number of antibiotic resistance pathogens has increased, always looking for an alternative drug that is considered safer.

II. MATERIAL AND METHODS

Collection of plant materials: The plants materials source obtained from Calendula officinalis (Marigold) flowers and Citrus sinensis (Orange) fruits peel were collected from local area of Betul district, Madhya Pradesh, India, in well cleaned and sterilized plastic ware.

Sample preparation for extraction: The collected material was cleaned under running tap water to remove dust particles on the surface of the plant material, and dried in shade for 10 days to remove the moisture present in the material, dried samples by a mechanical grinder is crushed so that the tissue is made homogeneous powder, and passed through a 40 mesh sieve. The collected powder of sample 100 g weights take on the cellulose paper and make the thimble.[14-16]

Soxhlet extraction: The powder obtained from the dried matter of plants was mixed with 200 ml of each of the solvents used, such as chloroform (Chl), ethanol (Eth.), methanol (Met.) and water (Watt) with a weight of 100 g, for three cycles sequentially. Phytochemicals were extracted separately using a Soxhlet extractor. Solvents were recovered at a temperature higher than the boiling point of the solvent, approximately 150 ml, and the solvents were collected in new flasks along with the remaining extract samples and the solvents were evaporated using a water bath apparatus, allowing the phytochemicals obtained in dry quantity.[14-16]

Antimicrobial screening: All obtained extracts were subjected to phytochemicals antimicrobial screening activity, tested through cellulose paper cut into small circular discs. For which the paper disc was saturated with various concentrations of 10-100 µg/ml of the previously extracted phytochemicals and the standard drug to determination of MIC.[17] After that NAM plates are prepared and the plates were kept open in air for 5 min. employing cultures of air-borne microorganisms and placing saturated discs on the surface of the NAM in plates and for antimicrobial assay the plates were incubated at 37 °C for 24 h in a BOD incubator. After that the antimicrobial effect was compared between the extracts of marigold flower, orange fruit peel and used as a positive control of standard antibiotic medicines.[14,15]

III. RESULTS AND DISCUSSIONS:

The extraction of phytochemicals from plants in specific types of selected organic solvents was primarily investigated based on the properties of organic solvents.[18] Organic solvents have a variety of properties such as hydrophobic or non-polar and hydrophilic or polar, depending on the nature of the solvents in which the extractive material is dissolved. To carry out this research, four different types of organic solvents, such as chloroform, ethanol, methanol, and water, were selected for the extraction of phytochemicals from two different plant species, known as the flowers of Calendula officinalis and fruits peel of Citrus sinensis was taken.[19,20] The chloroform, an organic solvent selected belonging to alkyl halides (R-X) groups, has non-polar properties for dissociated non-polar compounds. Two solvents ethanol and methanol which belong to the alcohol (R-OH) groups, have semi polar properties to dissolve semi polar compounds. A water (H-OH) group of solvent water was taken which is the polar property for polar compounds. Biomolecules such as a variety of phytochemicals exist in plants as polar and non-polar, and have a tendency to solubilize in specific solvents. The molecules found in phytochemicals have hydrophilic or hydrophobic properties according to their content.[21]

Organic solvents used to decompose compounds in varying amounts according to the properties of compounds that yielded 820 mg of phytochemicals extracted from COMF from 100 g of powder in chloroform and 780 mg of phytochemicals extracted from 100 g of powder of CSOFP, which was slightly lower than the phytochemicals extracted from COMF, and a difference of 40 mg was found in the amounts of phytochemicals derived from both plants extracts in chloroform. The solvent ethanol used yielded 670mg of phytochemicals from 100g of COMF powder and 860mg from 100g of CSOFP powder, slightly higher than COMF, and ethanol-based extracts from both plants and the difference of 190mg was found between phytochemicals derived from both plants extracts in ethanol, and methanol-based extracted phytochemicals yielded 850mg from 100g powder of COMF and 910mg from 100g powder of CSOFP, slightly higher than COMF, and the difference of 60mg was found between phytochemicals derived from both plants extracts in methanol. The extracts extracted from 100 g each of COMF and CSOFP powder by distilled water contained 840 mg and 540 mg of phytochemicals, which were found to be slightly less than COMF. A difference of 300 mg was found in the phytochemicals extracted from both plants in the water. Four different organic solvents were used, with a total phytochemicals obtained of 3.180 g and 3.090 g from 400 g of each COMF and CSOFP. There was a difference of 0.090 g in the amount of phytochemicals derived from both plant materials in the four solvents. The details are shown in Table 1.

The plant material compared to CSOFP, COMF was found to be a higher soluble compound of the plant material. The frequencies of extracts of

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phytochemicals soluble in organic solvents were found in their decreasing order thus methanol > water > chloroform > ethanol from COMF and methanol > ethanol > chloroform > water from CSOFP. The details are shown in Figure 1.

Table 1: Quantitative analysis of phytochemical extraction in organic solvents.

Organic sol.	COMF (mg)	CSOFP (mg)	Segregation (mg)
Chloroform	820	780	40
Ethanol	670	860	190
Methanol	850	910	60
Water	840	540	300
Total	**3180**	**3090**	**90**
Average	**795.00**	**772.50**	**147.50**

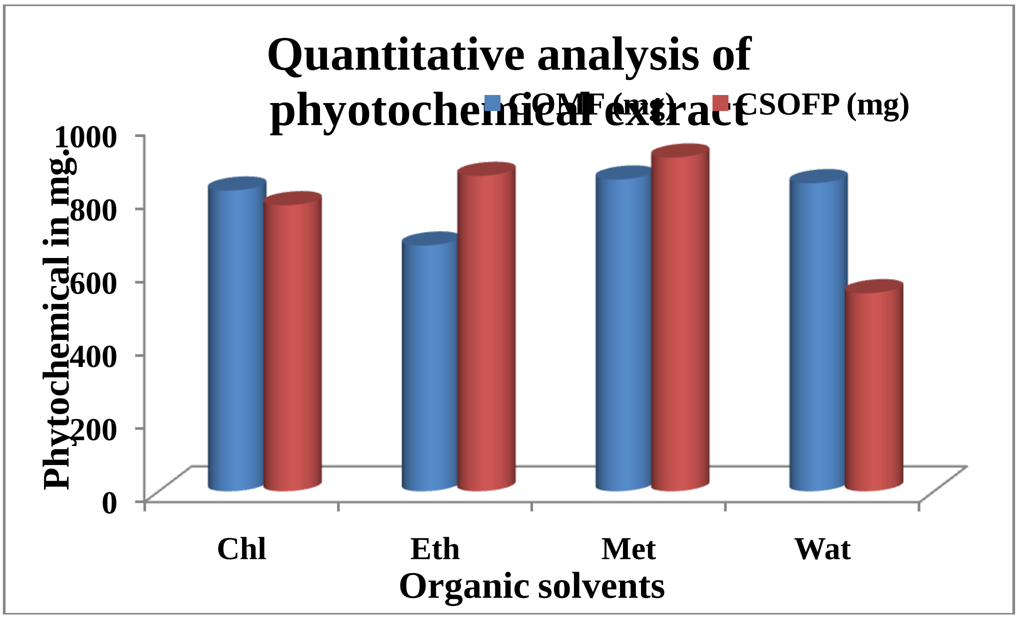

Figure 1: comparative analysis of phytochemicals extraction in organic solvents from two plants material COMF and CSOFP.

In this investigation the antimicrobial activity of COMF and CSOFP was determined against air-borne microorganisms by disc diffusion methods, absorbing the extracted samples into discs, in which two different plates A and B showed the antimicrobial activity of the extracted phytochemicals, the shown as growth inhibition activities. The details are shown in Figure 2. Antimicrobial active compounds were found in phytochemicals extracted from both resources of plant material. The percentage amount of phytochemicals extracted from COMF in four types of organic solvents was found to have the efficiency of determination of antimicrobial effect in percentage according to their frequency in increasing order such as 22.43% water, 25.00% chloroform, 26.10% ethanol and 26.47% methanol. Phytochemicals extracted from COMF in organic solvents were shown to exhibit highly antimicrobial activity of methanol > ethanol > chloroform > water soluble phytochemicals. The efficiency of antimicrobial effect in percentage according to their frequency in 21.37% water, 22.18% chloroform, 26.11% ethanol, 30.24% methanol was found to be similar to COMF in phytochemical compounds from CSOFP. The details are shown in Figure 3.

Comparative antimicrobial effects of phytochemicals extracted from both plants were found in 52% of COMF and 48% of CSOFP, whereas from a total of four organic solvents. The details are shown in Figure 4.

The rate of inhibition of the growth of microbes by phytochemicases extracted from both COMF and CSOFP plants was considered to be 100% of zone inhibition of microbes compared with (SM) standard medicine. The zone inhibition

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effects on microbes compared to SM to COMF plant samples were found to result from phytochemicals extracted in 52.71% chloroform, 55.04% ethanol, 55.81% methanol, 47.29% water. The zone inhibition effect of CSOFP on microbes against SM was found to result from the extracted phytochemicals in 42.29% chloroform, 50.39% ethanol, 58.14% methanol, and 41.09% water. The details are shown in Table 2 & Figure 5.

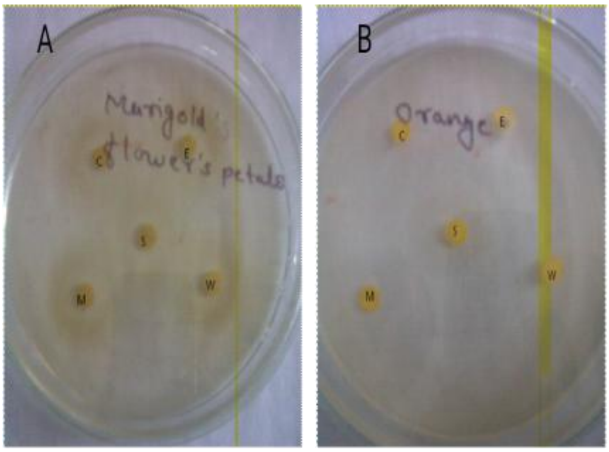

Figure 2: Optimization of antimicrobial effects shown the growth inhibition by saturated discs of SM and plants extract (A) COMF, (B) CSOFP.

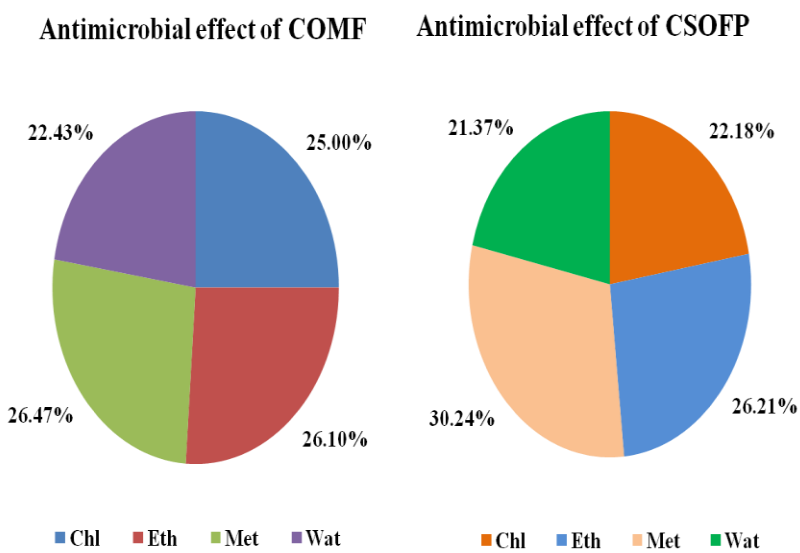

Figure 3: the individual antimicrobial frequency of phytochemicals extracted in organic solvents (Chl= chloroform, Eth= ethanol, Met= methanol, Wat= distilled water) from COMF and CSOFP.

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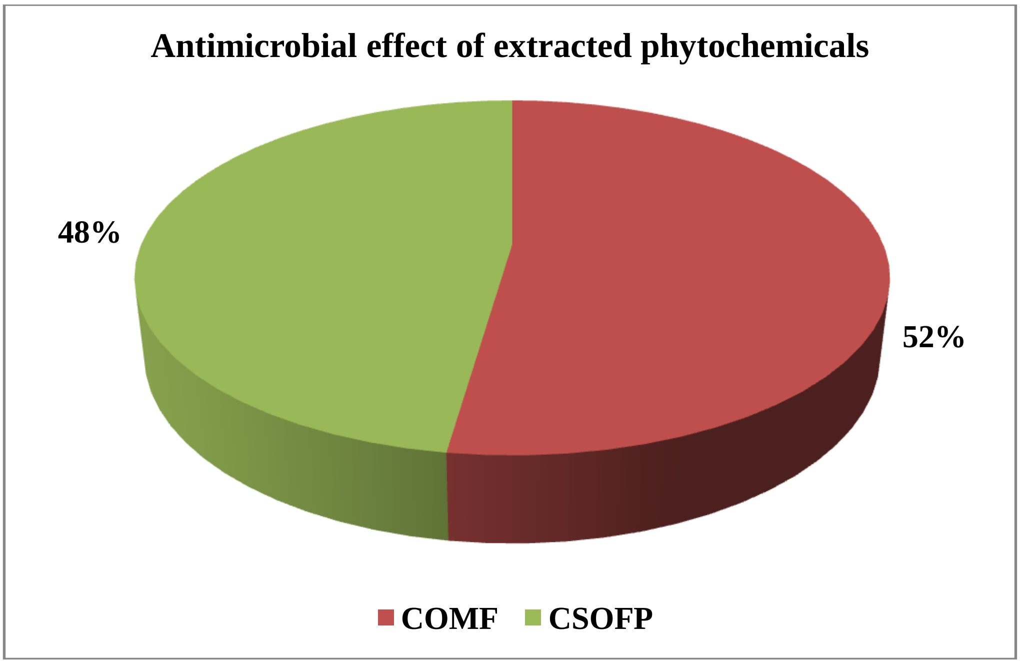

Figure 4: Comparative analysis of antimicrobial effects of COMF and CSOFP.

Table 2: The zone inhibition effects determination in percentage based analysis of phytochemicals of COMF and CSOFP against with SM (standard medicine).

	SM	COMF				CSOFP			
		Chl	Eth	Met	Wat	Chl	Eth	Met	Wat
Inhibited zone in (%)	100	52.71	55.04	55.81	47.29	42.64	50.39	58.14	41.09

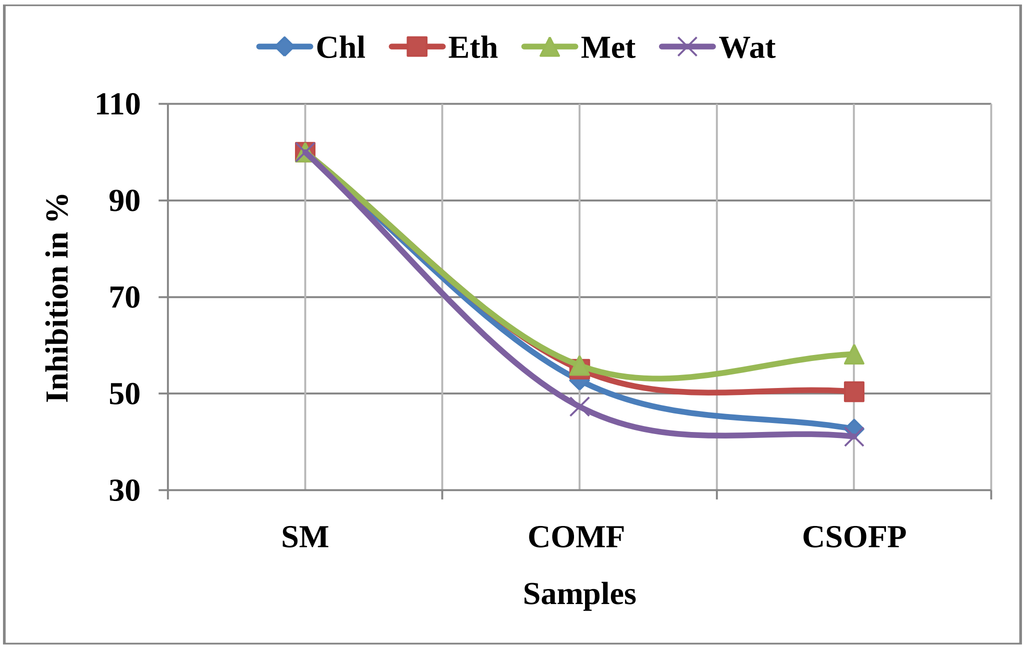

Figure 5: The zone inhibition effects determination in percentage based analysis of phytochemicals of COMF and CSOFP against with SM (standard medicine).

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The determination of MICs of plant extracted phytochemicals was obtained from COMF plant material in four selected organic solvents and compared with SM among air-borne microbes. The MIC of the chloroform based extracted phytochemicals was found to be approximately 90 µg/mL, which was equivalent to 50 µg/mL of the SM, the growth inhibition zone that surrounds the disc was found to have a radius of 1.3 cm. The ethanol based extracted phytochemicals were found to have an MIC of 60 µg/mL, which was found to equivalent 10 µg/mL of SM, and was found 0.8 cm radius of the disc surround zone of growth inhibition of microbes. The MIC of the methanol based extracted phytochemicals was found at 60 µg/mL, which was equivalent to 20 µg/mL of the SM, and was found in the 0.9 cm radius of the growth inhibition zone that surrounds the disc. The MIC of the distilled water based extracted phytochemicals was found approximately at 80 µg/mL, which was equivalent to 20 µg/mL of the SM, and was found in the 0.9 cm radius of the growth inhibition zone that surrounds the disc. The details are shown in Figure 6.

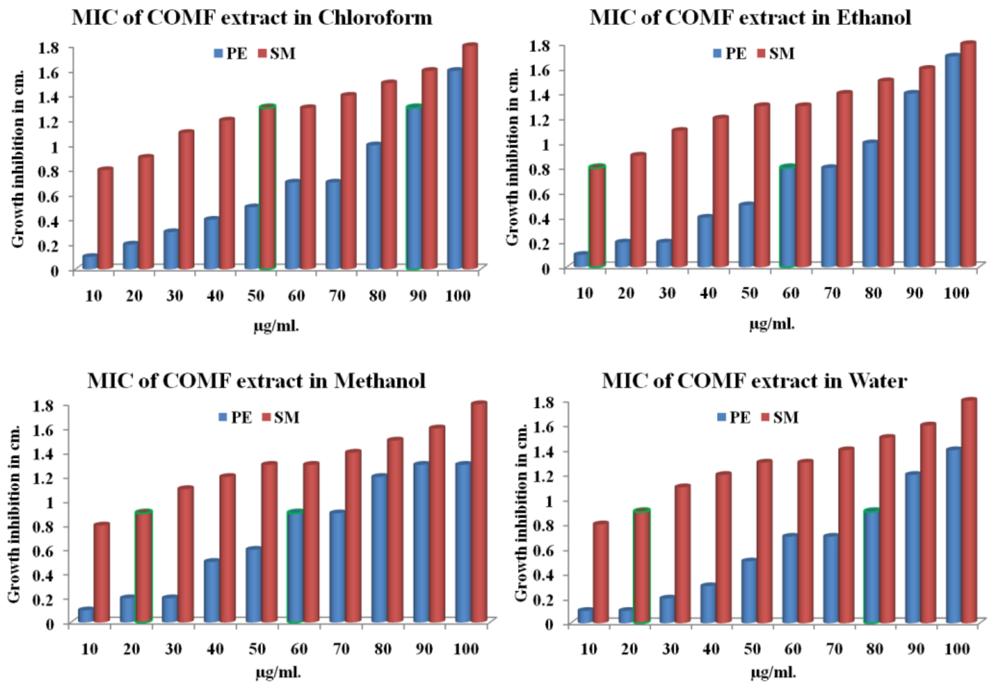

Figure 6. The estimation of MIC of COMF extracts in four organic solvents as chloroform, ethanol, methanol, and distilled water.

The determination of MICs of plant extracted phytochemicals was obtained from CSOFP plant material in four selected organic solvents and compared with SM among air-borne microbes. The MIC of the chloroform based extracted phytochemicals was found to be approximately 80 µg/mL, which was equivalent to 10 µg/mL of the SM, the growth inhibition zone that surrounds the disc was found to have a radius of 0.8 cm. The ethanol based extracted phytochemicals were found to have an MIC of 80 µg/mL, which was found to equivalent 20 µg/mL of SM, and was found 0.9 cm radius of the disc surround zone of growth inhibition of microbes. The MIC of the methanol based extracted phytochemicals was found at 60 µg/mL, which was equivalent to 10 µg/mL of the SM, and was found in the 0.8 cm radius of the growth inhibition zone that surrounds the disc. The MIC of the distilled water based extracted phytochemicals was found approximately at 80 µg/mL, which was equivalent to 10 µg/mL of the SM, and was found in the 0.8 cm radius of the growth inhibition zone that surrounds the disc. The details are shown in Figure 7.

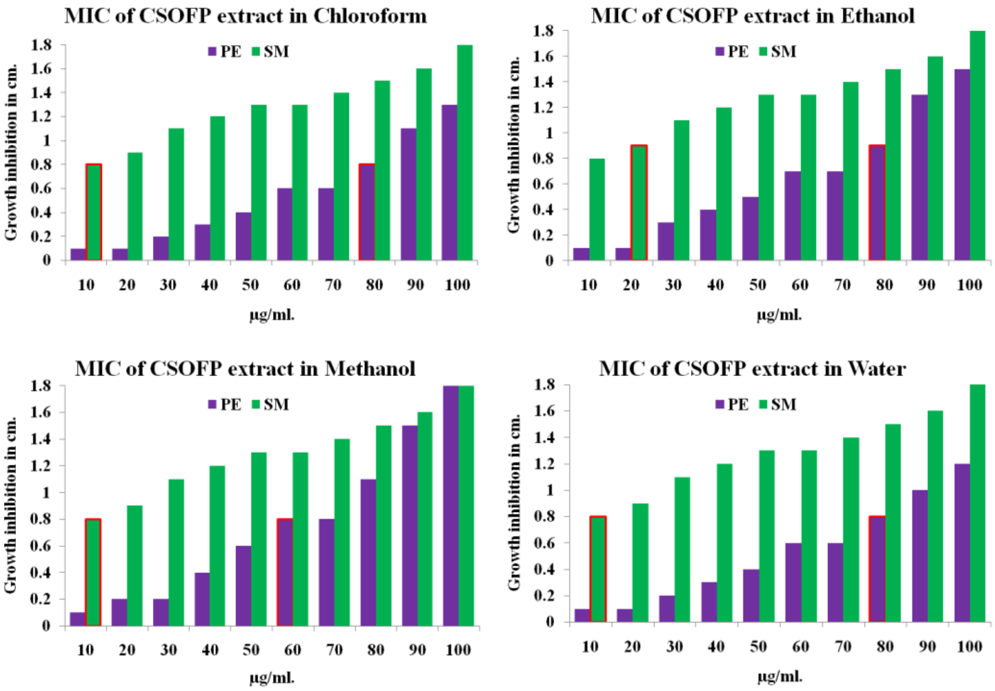

Figure 7. The estimation of MIC of CSOFP extracts in four organic solvents as chloroform, ethanol, methanol, and distilled water.

IV. CONCLUSIONS

The focus has always been on creating useful products from waste materials from selected plant materials such as COMF and CSOFP and these wastes are no exception. Appropriate methods should be adopted to utilize them for conversion into value added products. Manufacture of useful products such as medicine from fruit and flower waste can improve the overall economics of processing units in the pharmaceutical sector. COMF and CSOFP are rich in nutrients and contain several phytochemicals that have been shown to have antimicrobial activity; Efforts to reduce waste during the production process and recover the valuable product significantly reduce the amount of waste, as well as promote the environment. Both COMF and CSOFP are very beneficial to make and use in beauty creams to protect the skin from infection with air borne microorganisms. This study investigates the antimicrobial activity and essential scientific basis for its use by determining the chemical constituents of COMF and CSOFP, as well as the yield percentage of phytochemicals.

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