

# *In vitro* Evaluation of the Synergistic Antimicrobial Effect of *Boswellia Sacra* and *Nigella Sativa*, Essential Oil on Human Pathogenic Microbial Strains

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## ABSTRACT

**Objective:** The aim of the research work was to assess out the antimicrobial activity of *Boswellia Sacra* and *Nigella Sativa* essential oils. Essential oils are aromatic oils extract from vegetative as well as reproductive organs of plants such as stem, bark, root, flowers, fruits, seeds etc. The essential oils are a rich source of biologically active components and are shown to possess antibacterial, antifungal, antiviral, insecticidal, anti-helminthic and antioxidant properties.

**Methods:** Agar-Disc Diffusion Method And Preparation of Microbial Cultures:  $3 \times 10^5$  CFU/ml and Determination of minimal inhibitory concentration (MIC). In recent times, there is an extensive interest in essential oil due to the emergence and spread of new drug resistant human pathogens to existing antimicrobials. The coming out of medicine conflicting pathogens is one of the most vital threats to active treatment of bacterial disease.

**Result:** The result of antibacterial screening by agar disc diffusion method (Table-1) indicates that highest zone of inhibition was shown by the oil *Nigella sativa* for *E. faecalis* 45 mm/2.5  $\mu$ l and lowest for *Salmonella typhi* 14.5mm/2.5  $\mu$ l. The *Boswellia sacra* oils highest zone of inhibition for *Staphylococcus aureus* 24mm/2.5  $\mu$ l and lowest for the *Klebsiella pneumonia* 13mm/2.5  $\mu$ l. The mode of action of essential oils likely involves quite a lot of targets in the cell due to great number of active components and also their hydrophobicity enables them to partition in the lipids of the cell membrane, rendering them permeable and leading to leakage of cell contents.

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**Conclusion:** Although, both the oils were found to be effective in inhibiting pathogens to varying degrees to the tested organisms, the black cumin oil is found to be more effective than frankincense. Among the pathogens, *E. faecalis* was highly susceptible to both the oils. When both oils were used in combination, they have shown strong synergistic effect against all the pathogens tested in the present.

**Keywords:** Essential oils, Pathogenic microbial strains, Synergistic effects, MIC.

## INTRODUCTION

Based to the assorted health organization surveys such as WHO, almost 70-80% populations living in the developing countries rely almost solely on conventional medicine for their basic health care need. Investigation of the chemical constituents of the plants and pharmacological test may endow with us the basis for developing the progress of new agent's<sup>1</sup>. The importance of conventional medicines in solving the best of health problem solutions are invaluable on a global pharmaceutical market. Natural products have been a significant source of marketable medicines and drug source. Nearly 61% of drugs marketed worldwide can be outlined to natural products. Finding on medicinal plants has exaggerated and information on these plants has been exchanged. This research will go a long way in the scientific discovery of therapeutic plants for the assistance of man and is likely to diminish the belief on artificial drugs. The problem of microbial confrontation, resistance and degenerative diseases are growing and the stance for the use of synthetic drugs without unsympathetic effects in the future is still doubtful<sup>3</sup>. Medicinal plants are important therapeutic aids for various ailments and the use of those that are native to India in various traditional system of medicine are awe inspiring. The improved interest in alternative natural substances is driving the

research community to find novel uses and applications of these plants<sup>2</sup>.

The genus *Boswellia* has in the region of 20 species occurring in the arid regions spanning from West Africa to Arabia and south to the northeast region of Tanzania. In totalling, its species have been found in India and Madagascar. The genus is centered in northeast Africa, where approximately 75% of the species are endemic to the area<sup>4</sup>. Affiliates of this genus are trees or shrubs that are described as having outer barks that peel in parchment flakes, a greenish inner bark, watery aromatic resins, and wood with a milky latex. Frankincense, or olibanum, is the oleogum resin that is harvested from several different trees belonging to the genus *Boswellia*<sup>13</sup>. The word frankincense is derived from the ancient French name "frankincense," meaning "pure incense." Frankincense is also known in Arabic as "luban," which means "white" or "cream;" in Greek as "libanos;" in Ethiopia as "etan". Olibanum (frankincense) has been used as incense since ancient times. In recent years, it has been important in the preparation of cosmetics and perfumes. In addition, olibanum has anti-inflammatory, sedative, anti-hyperlipidemic, and antibacterial activities in Unani (Islamic) and Chinese traditional medicines. For at least 5000 years, olibanum has been an important trade

material for the civilizations located in North Africa and the Arabian Peninsula<sup>5</sup>.

The ethnobotanical literature reveals it as a versatile medicinal plant used to treat different human and livestock ailments. It has been reported as anti-inflammatory, analgesic, hypolipidemic activities, antimicrobial, diuretic, anti-plaque, astringent, aphrodisiac, and anti-pyretic. *Boswellia sacra*. Have great medicinal uses in the healing of toothache, bronchitis, tumors, gonorrhea, piles, leucoderma, scurvy, scabies and boils. These species contain important phyto-constituents such as, vitamin C, trimethylamine, tannins, cyanogenic glycosides, alkaloids, saponins and salts mostly as chlorides. The oil obtained from the seeds is used in painful rheumatic infection and after child-birth, seed fat of *Boswellia sacra* can be used for making candles and soap<sup>6</sup>. This research discusses the current knowledge of synergistic antibacterial effects on the seven human pathogens. *Nigella sativa* L. (Ranunculaceae family), known commonly as “black cumin”, is an herbaceous plant that grows in Mediterranean countries. Quite recently, many biological activities of *Nigella sativa* L. seeds have been reported, including: antioxidant, anti-inflammatory, anticancer and antimicrobial. In this study seed oil from *Nigella sativa* L. have been tested for antibacterial activity<sup>11</sup>. Mainly of the bacterial pathogens are resistant to existing synthetic antibacterial agents demanding an increasing effort to seek effective photochemical as antibacterial agents against such pathogens. *Nigella sativa* L. (black cumin) seeds play a crucial role in folk medicine and some of its major constituents are reported to be pharmacologically selectively active<sup>12,19</sup>.

The study was designed and imposed to examine the antimicrobial activities of essential oils of these plants by minimum inhibitory concentration (MIC) and the disc

diffusion methods. Essential oil (EO) samples of *Nigella sativa* and *Boswellia Sacra* were selected to determine antimicrobial activity against a wide range of disease borne gram-positive and gram-negative bacteria. Oils procured and were evaluated for their antimicrobial potential against seven bacterial strains (*Klebsiella pneumonia*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *E. faecalis*, *Salmonella paratyphi*, *Salmonella typhi*). EOs and their components show proficient activities against many disease-borne pathogens and spoilage microorganisms when tested *in vitro*<sup>14</sup>. Use of combinations of EOs and their isolated components are thus new approaches to increase the efficacy of EOs, taking benefit of their synergistic and combinational additive effects. The purpose of this research is to offer an overview on the antimicrobial efficacy of these combinations and to find the MIC standards. Both essential oils when used together exhibited higher MIC (Minimum inhibitory concentration) values towards all seven micro-organism especially, *E. faecalis* than their respective individual concentration. These results suggested the occurrence of synergism effects between the different oil constitutes a higher rate of antibacterial activity<sup>7</sup>. The study was carried out with an objective to investigate the, antimicrobial activity of *Nigella sativa* and *Boswellia Sacra* oil against seven human clinical isolates either alone or in combination. As microorganism are becoming resistant to present day antibiotics, our study focuses on antimicrobial activity and future prophylactic potential of this essential oil and to find out the minimum inhibitory concentration of seven human clinical isolates<sup>18</sup>.

## MATERIALS AND METHOD

### Source of oil

Commercially available oil of *Boswellia Sacra* and *Nigella sativa* from AL-BARAKH Saudi Arabia. As the oil is not been available in India, therefore it's been procured from inter country.

### Source of pathogen

Seven bacterial cultures *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* and *Klebsiella pneumoniae* were used in the present study; all the tested strains were obtained from Sagar Apollo Hospital, Karnataka, India. Bacterial strains were cultivated in nutrient broth (HiMedia, M001) at 37°C and preserved on nutrient agar slants at 4°C.

## METHODS

### Agar-Disc diffusion method and preparation of microbial cultures: $3 \times 10^5$ CFU/ml

The evaluation was conducted by agar disc diffusion technique. About 25 to 30 ml of Nutrient agar medium was poured in the sterilized petridishes and allowed to solidify at room temperature. Bacterial strains were cultured overnight in Nutrient agar (HiMedia, Mumbai) at  $37 \pm 2^\circ$ . Overnight grown culture of microorganisms was used for inoculums preparation. A loopful of isolated colony was inoculated in 4ml of peptone water (HiMedia, Mumbai) at 37°C for 2h. The turbidity of resulting suspension was compared to 0.5 McFarland turbidity standards<sup>15</sup>. The level of turbidity was corresponding to approximately  $3.0 \times 10^5$  cfu/ml. The Nutrient Agar media (HiMedia, Mumbai) solidified, was then inoculated with microorganism suspended in peptone water, using sterile swab stick, standardized inoculate of each isolate was swabbed onto the surface of Nutrient Agar

in separate petridishes. Discs of the extracts were placed to the surface of the inoculated media<sup>8</sup>. The plates were inverted and allowed to stand for 30 minute for the oil to diffuse into the agar after which the plates were incubated. The Petri dishes were incubated for 24 h at  $37 \pm 2^\circ\text{C}$  for bacteria. The antimicrobial activity was calculated by measuring the diameter of zone of inhibition in millimeters around the well of the discs, shown in (table -1, fig-1) below<sup>9</sup>. However the robust combinational effect of the oil can be seen more effective in the entire microorganism such as *E. coli*, *S. typhi*, *S. aureus*, *E. faecalis*, *K. pneumoniae*, *P. aeruginosa* and *S. para typhi*. [All the microbial strains are highly sensitive to Ampicillin with Clavulanic acid proving their pathogenicity]<sup>16</sup>.

### Determination of minimal inhibitory concentration (MIC)

A minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that inhibits the growth of a microorganism after 18 to 24 h. The oils that showed antibacterial activity by agar well diffusion method were subjected to serial micro broth dilution technique to determine their minimum inhibitory concentration by using turbidimetric evaluation<sup>10</sup>. In this MIC was determined by the liquid dilution method, dilution series were set up with 180µl of nutrient broth medium, to each microtiter well 10µl of standard suspension of bacterial colony was added and 10µl of diluted oils was added and incubated at 37 for 24 hours<sup>17</sup>. The lowest dilution which did not show any growth for the tested bacteria after microscopic evaluation was determined as MIC. Based on the evaluation *S. typhi*, *E. faecalis*, *K. pneumoniae* is showing the MIC at 1: 200 dilutions while the one which is being inhibited least is *S. paratyphi* that is at 1:180 dilutions. The below mentioned table

-3 shows the individual minimal inhibitory concentration (MIC) of the microorganisms.

## RESULT AND DISCUSSION

*Nigella sativa* and *Boswellia sacra* have the immense medicinal value. The tested bacterial strains showed different pattern of inhibition zone. Readings were recorded in tabular form (Table 1). The oil of *Nigella sativa* showed more antimicrobial activity than *Boswellia sacra*.

The result of antibacterial screening by agar disc diffusion method (Table-1) indicates that highest zone of inhibition was shown by the oil *Nigella sativa* for *E. faecalis* 45 mm/2.5 µl and lowest for *Salmonella typhi* 14.5mm /2.5 µl. The *Boswellia sacra* oils highest zone of inhibition for *Staphylococcus aureus* 24mm/2.5 µl and lowest for the *Klebsiella pneumonia* 13mm/2.5 µl.

Robust combinational effect were observed, when the oils were used in combination for the *E.coil*, 30mm, *Staphylococcus aureus* 35mm, *P. Mirabilis* 34mm, *Salmonella paratyphi* 35mm and *K. pneumonia* 23mm effect (Table-2).

A more generally accurate method of assessment is the broth dilution technique. In this study, therefore, the broth dilution method was used in determining the activities measured as MIC by turbidimetric evaluation. The range of MIC values for all the microbial strains correlated well with the results obtained by using the agar disc diffusion method.

The minimum inhibitory concentration when used in combination is highest for the *Enterococcus faecalis* (MIC-1:200), *Salmonella typhi* (MIC-1:200) and *K. pneumonia* (MIC-1:200) dilution and lowest for the *Staphylococcus aureus* that is (MIC-1:170) dilution (Table-3). Therefore due to the antimicrobial activities of these plants there are several reasons that people use plants for medication. This includes progress

of health following herbal treatment, low cost of the drugs, non availability of synthetic drugs particularly in the countryside areas, where available were either fake or expired drugs and in some cases the people are more familiarized to and comfortable with traditional healing.

## CONCLUSION

In this context, two essential oils from traditional medicinal plants, black cumin (*Nigella sativa*) and Frankincense (*Boswellia Sacra*) were used alone or in combination to assess their antibacterial efficacy against both gram positive and gram negative bacterial clinical isolates. Among the pathogens, *E. faecalis* was highly susceptible to both the oils. When both oils were used in combination, they have shown strong synergistic effect against all the pathogens tested in the present study.

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## REFERENCES

1. Analysis of Microbial Contamination in Food Grade Samples at the Industrial Production levelwaghode S.M. and A.M. Garode, *I. Res. J. Biological Sci.*, 2(2), 78-80 (2013).
2. Isolation and Characterization of Multi drug Resistant Super Pathogens from soil Samples Collected from Hospitals Prasad Chandan, Mishra R.P., Ali Asif, Gangwar V.S. and Chand Shweta, *Res. J. Recent Sci.*, 2(ISC-2012), 124-129 (2013).



3. Emergence of multi-drug-resistant Klebsiella pneumonia in Neonatal Intensive Care Units: concern about antimicrobial policies Shah Manisha N. and Desai Pratibha B., *Res. J. Recent Sci.*, (ISC-2011), 275-280 (2012).
4. Eloff J.N., A sensitive and quick microplate method to determine the minimal inhibitory concentration of the plant extracts for bacteria, *Planta Medica*, 64, 711-713 (1998).
5. Cowan, M.M. (1999) Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 12: 564-82.
6. Eloff J.N., Which extract should be used for the screening and isolation of antimicrobial components from plants, *J. Ethnopharmacology*, 60, 1–8 (1998).
7. Epino P.B. and Chang F., Insecticidal activity of *Annona squamosa* L. Seed extracts against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). *Philippine Entomologist*, 9, 228-238 (1993).
8. Eloff, J.N. (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of the plant extracts for bacteria. *Planta Medica* 64: 711-713.
9. Rao, T.A. and Chakraborti, S. (1996) Salvadoraceae *Salvadora persica* L. var. *indica* (Wight) and *Salvadora persica* L. Var *tuticornica*. *Fasc. Fl. India*, 22: 9.
10. Evaluation Of The Combinational Antimicrobial Effect Of *Prunus Persia* And *Annona Squamosa* Seeds Methanolic Extract On Standard Microbial Strains, *Global Journal Of Bioscience And Biotechnology*, G.J.B.B., Vol. 2 (4) 2013: 571-575, ISSN 2278 – 9103, 1Anup Baranwal, 1Shikha Arora, 1Rakesh Kumar G, 1B.K.V.R.Prasad, 2Jeevena Praharsha, 2Aamir Javed 3Md.Hidayatullah, 4Neda Sanobar.
11. Suresh K, Manoharn S, and Blessy D. Protective role of *Annona squamosa* Linn bark extracts in DMBA induced genotoxicity. *Kathmandu University Medical J.* 2008; 6(3):364-369.
12. Irobi O.N., Young M. and Anderson W.A., Antimicrobial activity of annatto (*bixaorellana*) extract, *Int j Pharma*, 38- 90 (1996).
13. Synergistic Antibacterial Effect Of Essential Oils Of *Nigella Sativa* And *Salvadora Persica* On Human Pathogens Agurumurthy, K.T., Achandan, N, A\*Aamir Javed, Aashwini, L.S., Bmohd Nadeem Khan, Cshazia Shaheen & Danup Baranwal. *International Journal of Advanced Biological Research I.J.A.B.R.*, Vol. 3(4) 2013: 567-571.
14. Janssen, A. M., Scheffer, J.J.C. and Baerheim-Svendsen, A. (1987) Anti-microbial activity of essential oils: a 1976-86 literature review. *Planta Medica*, 53: 395-398.
15. Mcclintock E. Arecaceae palm family, The Jepson Manual: [http://ucjeps.berkeley.edu/cgi-bin/get\\_JM\\_treatment.pl? Phoenix+dactylifera](http://ucjeps.berkeley.edu/cgi-bin/get_JM_treatment.pl?Phoenix+dactylifera) (2007).
16. Dickert, H., Machka, K. and Braveny, I. (1981). The uses and limitations of disc diffusion in the antibiotic sensitivity testing of bacteria. *Infection* 9: 18-24.
17. Kirtikar, K.R. and Basu, B.D. (1968) Indian medicinal plants Lalit Mohan Basu, Allhabad, India. (Vol. I and II).
18. Dua'a A. Abo Al-Samh' and Nasir H. Al-Bagieh (1996). A study of the antimicrobial activity of the miswak ethanolic extract *in vitro*. *Biomedical lethers*. 53 225-238.
19. Evaluation Of The Combinational Antimicrobial Effect Of *Annona Squamosa* And *Phoenix Dactylifera* Seeds Methanolic Extract On Standard Microbial Strains , Javed Aamir\*<sup>1</sup>, Annu Kumari<sup>2</sup>, Mohd. Nadeem Khan<sup>3</sup> and Siva Kumar Medam<sup>4</sup> 1\*, 2, *International Research Journal of Biological Science*. Vol. 2(5), 68-73, May (2013) ISSN 2278-3202.

**Table 1.** Zone of inhibition organism name

Organism	Oil 1: <i>Nigella sativa</i> Zone of inhibition (mm)	Oil 2: <i>Boswellia Sacra</i> Zone of inhibition (mm)	Mixed: Oil1 and Oil2 Zone of inhibition (mm)
	2.5 µl	2.5 µl	2.5 µl
<i>E. coli</i>	19	14	30
<i>S. typhi</i>	14.5	16	23
<i>S. aureus</i>	21	24	35
<i>E. faecalis</i>	45	22	45
<i>P. mirabilis</i>	20	22	34
<i>S. paratyphi</i>	22	16	35
<i>K. pneumonia</i>	15	13	23

**Table 2.** Control OD Vs MIC OD

Pos.	Organism	Quality	Rack-N	<i>N. sativa</i> (1)		<i>Boswellia Sacra</i> (2)		Mix (1+2)	
				OD-C	OD-Mic	OD-C	OD-Mic	OD-C	OD-Mic
1	<i>E. coli</i>	☺	R1-1	0.55	0.48	0.55	0.50	0.53	0.51
2	<i>S. typhi</i>	☺	R1-2	0.31	0.27	0.32	0.30	0.31	0.28
3	<i>S. aureus</i>	☺	R1-3	0.41	0.38	0.41	0.38	0.40	0.39
4	<i>E. faecalis</i>	☺	R1-4	0.80	0.72	0.81	0.79	0.82	0.79
5	<i>P. mirabilis</i>	☺	R1-5	0.80	0.70	0.81	0.79	0.80	0.79
6	<i>S. paratyphi</i>	☺	R1-6	0.32	0.30	0.32	0.30	0.31	0.29
7	<i>K. pneumonia</i>	☺	R1-7	0.85	0.75	0.85	0.79	0.80	0.79

OD-Optical Density, MIC-Minimum inhibitory Concentration, R1-7—Rack Position in Spec Analysis.

**Table 3.** MIC oil efficiency oil dilution

Organism	<i>Nigella Sativa</i> (1)	<i>Boswellia Sacra</i> (2)	NS+BS
<i>E. coli</i>	160	120	190
<i>S. typhi</i>	110	140	200
<i>S. aureus</i>	130	100	170
<i>E. faecalis</i>	120	140	200
<i>P. mirabilis</i>	150	120	185
<i>S. paratyphi</i>	150	120	180
<i>K. Pneumonia</i>	120	140	200

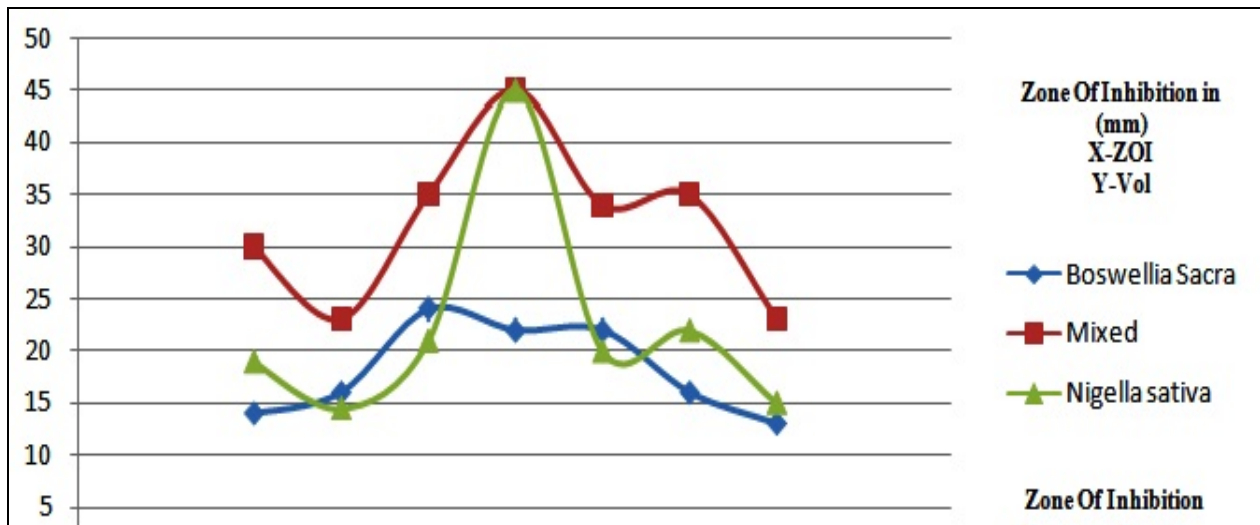


Figure 1.

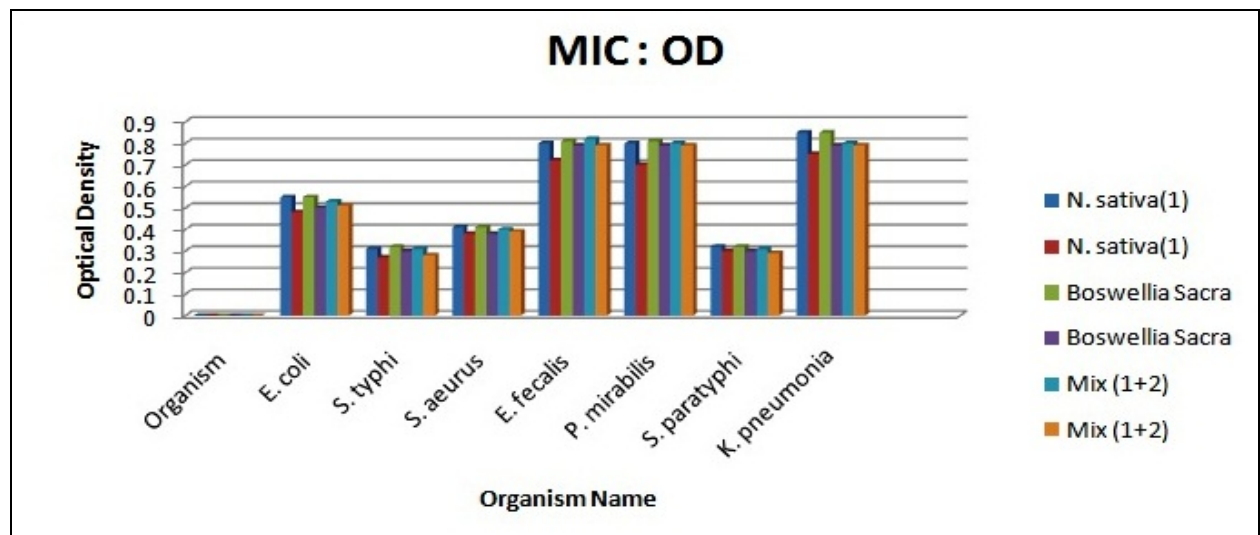


Figure 2.