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Detection and Analysis of Active Compounds in *Nigella sativa and Senna alata* through Biological Methods

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Abstract: The study is centered on compounds derived from Nigella sativa and Senna alata; extraction, identification and quantification methods of which are the focus of this study together with a focus on newer analytical techniques. These plants are known for their healing properties, namely, bactericidal, anti-inflammatory and anti-oxidative activity. The study seeks to observe these compounds with a view of determining their capacity in the creation of therapies.

Keywords: Phytochemicals, Healing Herbs, Properties against Bacteria, and Reduce Inflammation.

1. Introduction

Nigella sativa and Senna alata are both applied within the conventional medicinal drug of diverse cultures. because of the numerous pharmacological results related to their bioactive compounds, they have garnered sizeable interest from scientists. studies into these compounds, their organic consequences, and ability healing packages is being approached thru a harmonious combo of traditional and medical strategies. .[1] Black cumin additionally called *Nigella sativa,* is a plant that has been recognized to the middle East and the regions lying to the South and North East of it for centuries. it's far preferred for its medicinal cost as a digestive aid, a booster of immunity as well as remedy of respiration sicknesses. The foremost energetic ingredients discovered in black cumin belongs to the institution referred to as the quinones and those encompass; thymoquinone, thymohydroquinone and dithymoquinone. these compounds have shown antioxidant, antimicrobial and anticancer potentials and consequently were the focus of lots studies. [2] Senna is higher called the candlestick plant, which grows within the tropic and turned into common in the traditional remedy structures of African, Asian and American areas. it's been applied as a shape of treatment due to its recovery properties which might be effective in addressing skin infections, fungal sicknesses, and gastrointestinal issues. *Senna alata* plant consists of anthraquinones derivatives such as Rhenium, aloe-emodin, and Kaempferol. All of those compounds have a bioactivity profile that includes antimicrobial, antiviral, antifungal, and antioxidant interest, in addition to consequences. The present day procedure of figuring out and describing the active compounds in *Nigella Sativa and Senna alata* involves using chromatography (HPLC and GC-MS), spectroscopy (UV-Vis and RMN), and a spread of organic exams. consequently, it's far important to behavior extra research on the chemical structures and organic sports of those molecules so that you can recognize their movement

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mechanisms and explore their potential medicinal applications in the destiny [5]. In this newsletter, current facts revealed on the active components of *Nigella Sativa and Senna Alata* might be disassociated with the pharmacological residences, staining strategies, comparative members of the family between the traditional makes use of and findings of new studies, and the purpose of establishing a bridge among folks makes use of and current technology, and additionally to inspire the following technology of research on the healing uses of these vegetation, this article intends to offer a comprehensive review of all fundamental aspects.

2-Materials and Methods

The *Nigella sativa and Senna alata* leaves used within the study had been received from licensed carriers. ultimately the plant material turned into washed with distilled water and then allowed to air dry it became then mechanically milled right into a high-quality powder. To make certain complete extraction of the numerous connections, the extraction technique made use of 3 solvents: ethanol, methanol and water solvents primarily based by means of their polarity [6], the typical ethanol extraction method consists of soaking 50g of powdered seeds or leaves in 500ml of ninety five% ethanol. The mixture turned into agitated in an incubator for 72 hours at room temperature. The final product was filtered the use of Whatman No. the solution became filtered using filter out paper after which concentrated using a low pressure rotary evaporator at a temperature of 40 °C. The ensuing pattern changed into then saved at a temperature of four °C for future using an extracting which include 80% methanol led to an extraction method that closely resembled the only defined above [7]. The plant fabric observed the same strategies that encompass soaking, incubating, filtering, attention and garage. To put together the aqueous extraction, add 500 ml of distilled water to 50 g of powdered fabric and permit it to stand for a while after which filter the solution thru Whatman No. 1. We passed the gel via a filter out to separate it into a filtrate and precipitate. The filtrate became then freeze-dried to produce a dry powder, which became stored at 4°C for future evaluation. the usage of those equations, the amount of extract yield turned into determined in line with the manner defined in[8]. To determine percentage yield possible weigh the dried extract and multiply by using the load of the original plant material and then multiply by way of one hundred. Yield in percentage is calculated as a result yield = weight of dried extract / weight of unique plant cloth x one hundred. The subsequent components is used in expressing productivity in chances; weight of dried extract/weight of unique plant cloth x 100. in the case of plants the first step worried in the evaluation of healing homes of the plant material is phytochemical screening this is executed with the purpose of identifying all of the physiologically active substances. on this phase the technique for the qualitative phytochemical evaluation for the extracts of Senna and Nigella sativa changed into defined following the protocols described in [9,10]. The *Nigella sativa and Senna* seeds were dried, finely floor, and saved in airtight packing containers for future use in the extraction procedure. Ten grams of each ground plant cloth have been in my opinion extracted with 100 ml of ethanol, methanol, and water. The phytochemical tests blanketed the following: 1- Alkaloids (Wagner's check): 2 ml of the extract were combined with Wagner's reagent (iodine in KI). A tremendous end result for alkaloids in a test could bring about the introduction of a reddish-brown precipitate. 1- Flavonoids

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(Shinoda check): 2 ml of the extract was blended with magnesium strips and focused HCL. it is important to mention that an item with a purple or red shade incorporates flavonoids. to check for saponins, milliliters of the extract were mixed with distilled water in a box, which was then tightly sealed and vigorously shaken to create foam. An extended and rigid formation of froth at some point of the take a look at confirms the presence of saponins.
0.1% Ferric chloride. the appearance of blue-black or green sediment method that tannins and 4-phenols are present (iron chloride take a look at). To behavior the check, 2 ml of the extract had been mixed with a small amount of 5% iron chloride solution. The lifestyles of phenols is indicated by using the presence of darkish blue or black colour. The salkovski take a look at for terpenoids worried blending 2 ml of the extract with chloroform and concentrated sulfuric acid. The presence of terpenoids signifies the advent of a reddish-brown boundary.
Chromatographic analysis: all of the techniques along with identity, isolation and quantification of the attention of physiologically energetic chemical compounds gift inside the plant extracts may be nicely completed the usage of contemporary chromatographic strategies. This element therefore goals at giving a brief description at the chromatographic analysis that turned into completed on *Nigella sativa and Senna alata* as well as the procedures that have been observed and the outcomes that have been received. pattern guidance as described by using [11] The raw extracts of *Nigella sativa and Senna* were dissolved in their suitable solvent (ethanol, methanol, or water) and in the end filtered through a zero.2 µm. clear out. Use a forty five µm membrane clear out to eliminate the debris. The extracted filters were then prepared for evaluation using Chromatography. consistent with B. [12], HPLC is described as • gear: HPLC system with a quaternary pump for easy transport of four solvents, an automatic air purger, and a UV-VIS detector. The cell phase used for the C18 reversed-segment column (250mm × 4) A particle length of five micrometers (6mm) became utilized. The solid bureaucracy had been obtained using solvent A, that's distilled water with 0 percentage. 1% NaNO solvent and zero.four% acetonitrile solvent B. 1 percentage NaNO solvent. covered: 1 glide fee. drift price of 20 mL/min, with detection at 254 nm for glycemic float. Injection quantity turned into 20µL and the column temperature changed into held at 30 °C. gas chromatography-mass spectrometry (GC-MS) as defined in reference [13] turned into using an electron ionization source and a capillary column measuring 30 meters in period and 0. 25 mm, zero film thickness. 25 micrometers), • Helium gasoline used as the carrier at a glide fee of 1 preferred liter in step with minute (SLPM). Injection temperature: 250 °C, flow price: zero mL/min. The oven temperature application is set at 60 °C for two min, followed with the aid of a ramp at 10 °C/min to 250 °C with a 10 min keep. The ion source temperature is 230 °C with a mass range being decided. It become important to analyze the composition of the biologically energetic compounds in Nigella sativa and Senna alata extracts so as to pick out their chemical profile and potential medicinal residences.
Antimicrobial pastime: This phase consists of details about the techniques used for checking out the antimicrobial properties, in addition to the effects of experiments carried out using *Nigella sativa and Senna alata* extracts on various bacterial and fungal traces. The microorganisms hired in the test include the following: *Staphylococcus aureus, Bacillus subtilis,*

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Escherichia coli, Pseudomonas aeruginosa (all of that are bacterial lines), as well as Candida albicans and Aspergillus niger (both of which might be fungal strains). in step with a recent have a look at by way of Umer et al [14], we prepared ethanolic, methanolic and aqueous extracts of *Nigella sativa and Senna alata* at concentrations of 50, a hundred, and 2 hundred mg/mL.

B.For nicely diffusion method as described by [15]

1. pattern guidance: each censu bacterial and fungal lines have been cultured in Sabouraud nutrient broth at the same time as each censu fungal and bacterial lines had been cultured in dextrose broth and incubated at 37 0C for 24hrs.
2. Agar plates: depending at the bacterial lines nutrient agar plates were used and in case of fungal lines Sabouraud dextrose agar plates were used.
3. pattern: Samples of bacterial suspensions were inoculated on the agar plates uniformly with a help of a sterile cotton tipped cotton bud.
4.Pozi formation: those wells of 6 mm diameter had been bored on agar plates respectively by a sterile cork circle.
5.Extract software: a hundred µl of every of the stated concentration of the plant extract was allotted to the respective properly.
6.Controls: high quality control; this turned into popular antibiotics for each of the organisms examined; bacteria changed into grown on ampicillin and fungi on ketoconazole poor manage; solvent used in extraction which consist of ethanol, methanol and water.
7. Incubation: It turned into further incubated at 37 °C for in the future in case of bacterial strains isolates and two days in case of fungal isolates.

C. consistent with [16], the scale of the inhibition zones surrounding each nicely was measured in millimeters (mm). The minimum inhibitory concentration (MIC) references have been decided using the broth microdilution technique. The extracts have been diluted and added to 96 nicely Microtiter plates, then microbial suspensions were introduced to the wells and the plates were incubated. The MIC changed into described as the minimum concentration of the extract that prevents the growth of microbes and the presence of seen boom. and antioxidant assessments: This passage outlines the strategies and findings of tests examining the antioxidant homes of *Nigella sativa and Senna alata* extracts, aimedtoward identifying their capacity therapeutic advantages as stated via [18]. To inhibit protein breakdown, 1 ml of plant extract (at concentrations of 50, one hundred, and 200 µg/ml) became combined with 1 ml of five% V/V alo-albumin and 1 ml of phosphate buffer solution (pH 6.four). The combination became left to incubate for 20 mins at a temperature of 37 tiers Celsius after which heated for 5 mins at a temperature of 70 tiers Celsius. The measurement of absorption took place at a wavelength of 660 nm. manage became finished using distilled water, while diclofenac sodium was used as the standard substance. carrying out a take a look at on two membranes: a healthy volunteer's blood was amassed and transformed right into a round red blood cellular at a attention of 10%. Suspension of cells in zero.5 ml of isosaline answer. After being incubated for half-hour at 37 ° C after which centrifuged, the absorbance of the top liquid become measured at 560 Nm. Distilled water has been utilized as the standard manipulate and for testing diclofenac sodium. The

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calculation for the hemolysis inhibition report is as follows: The components for the inhibition ratio is calculated via subtracting the witness antibody pattern from the manage antibodies after which multiplying the result via 100. The Hemm ratio is represented as the fraction of the control antibody. [19] consistent with [19], the 3-DPPH coil's effectiveness in neutralizing free radicals was tested through combining 1 ml of a zero.1 mmol DPPH solution in methanol with 1 ml of plant extract (at concentrations of fifty, one hundred, and 200 µg / ml), then incubating the mixture in darkness for 30 minutes and measuring the absorption at 517 nm. Methanol become hired as a reference, whilst ascorbic acid served as the usual, the radical decoloration take a look at defined in reference [20]. the novel arcation was produced and then diluted to a selected level of absorption. 1 milliliter of diluted answer became combined with 1 milliliter of plant extract (at concentrations of 50, 100, and 2 hundred µg/ml) and left to incubate for 30 minutes. The size of absorption was taken at 734 nanometers. Methanol served as manipulate while Trolox become used as the usual. The reason of the study became to assess the antioxidant results of *Nigella sativa and Senna alata* extracts, in addition to tuberculosis extracts, with the aim of figuring out their potential healing.

3-Results:

Initial analysis of the photosynthesis process showed that bioactive compounds were present in the extracts of both *Nigella sativa and Senna* plants. The results of the phytochemical screening are summarized in Table 1 and Figure 1.

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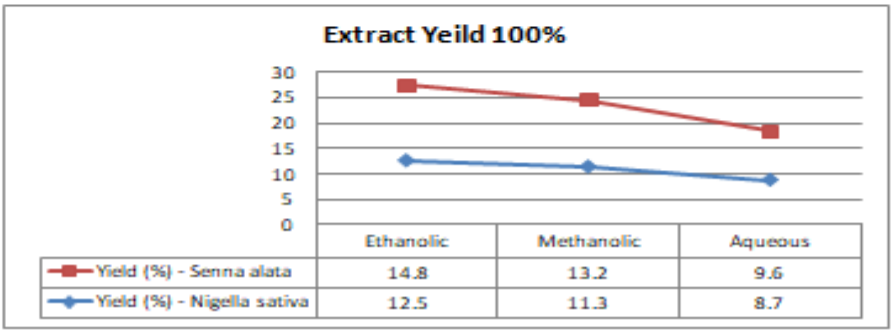
Table 1 displays the findings of the phytochemical screening.

Phytochemical	*Nigella sativa*	*Senna alata*
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Phenols	+	+
Terpenoids	+	+

(+) indicates the presence of the phytochemical., (-) indicates the absence of the phytochemical.

Figure 1: Extract Yield (%)



The principle chemical additives within the plant extracts have been recognized through the usage of high-performance liquid chromatography (HPLC) and gasoline chromatography-mass spectrometry (GC-MS) analyses. *Nigella sativa* turned into located to have thymoquinone, niglidine, and niglycine, while Senna alata contained aloe-emodin, rhein, and kaempferol.

The results of the HPLC analysis for *Nigella sativa* confirmed thymoquinone eluting at five.eight minutes, niglycine eluting at 12.3 mins, and niglycine eluting at 15.4 minutes. The exam of *Senna alata* discovered the presence of aloe-emodin at a retention time of 7.2 mins, rhein at a retention time of 13.6 minutes, and kaempferol at a retention time of 18.7 minutes.

The evaluation the use of gas chromatography-mass spectrometry detected unique compounds in Nigella sativa and Senna alata. Thymoquinone (Rt = eight.5 min, m/z 164), p-Cymene (Rt = 10.3 min, m/z 134), and thymohydroquinone (Rt = 12.1 min, m/z 150) have been identified in Nigella sativa. Senna alata changed into determined to contain chrysophanol (Rt = nine.four min, m/z 254), emodin (Rt = eleven.7 min, m/z 270), and quercetin (Rt = 14.9 min, m/z 302).

each samples confirmed a wide spectrum of antimicrobial pastime. *Nigella sativa* confirmed robust antibacterial consequences *against Staphylococcus aureus and Escherichia coli, even as Senna alata* displayed full-size antifungal activity against *Candida albicans*. The antibacterial properties of *Nigella sativa and Senna alata* extracts in display in discern (2) and Appendix 1

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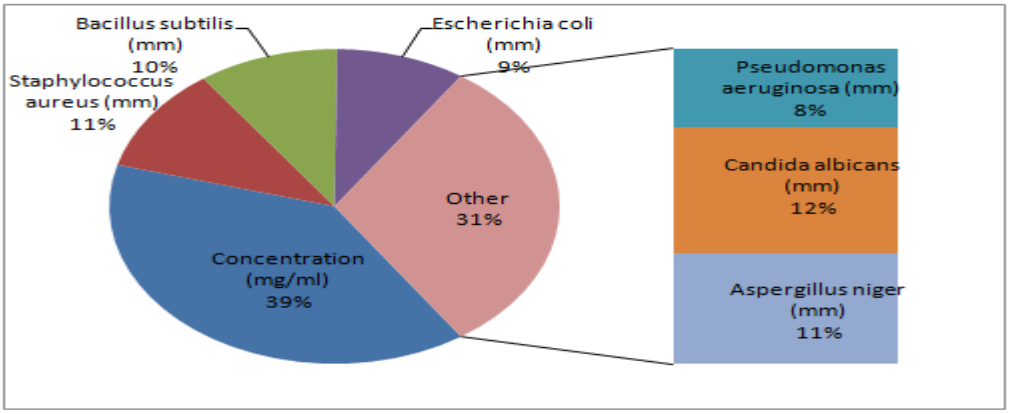

Figure (2) Aantimicrobial activity of *Nigella sativa and Senna alata* slant extracts against different microorganisms at different concentrations and extraction methods.

The minimum inhibitory concentration (MIC) values for *black seed and senna* extracts against several microorganisms show in Figure (3) and Appendix 2

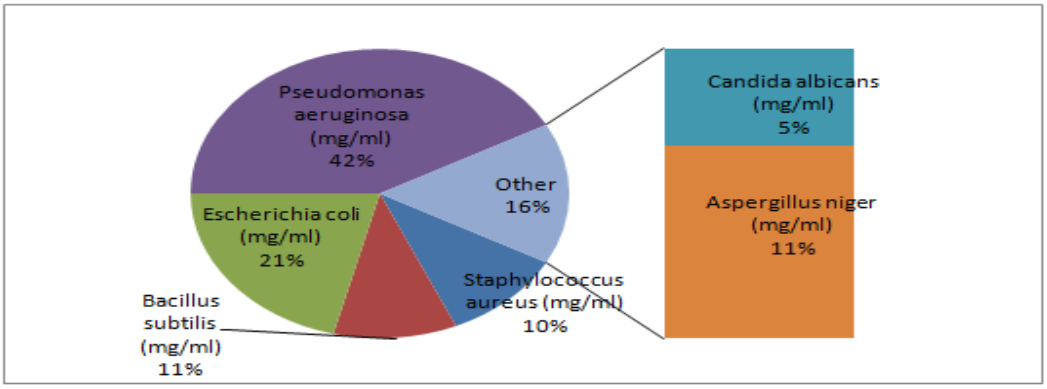

Figure 3: The minimum inhibitory concentration values indicate the lowest concentrations (in mg/ml) of the extracts at which microbial growth was inhibited for each microorganism tested.

Representative compounds demonstrated concentration dependent ability to suppress nitric oxide production in LPS induced macrophage, proving good anti-inflammatory activity. In the present investigation, both the extracts demonstrated significantly higher antioxidant activity in both DPPH and ABTS assays suggesting the higher free radical scavenging potential Appendix 3.

Nigella sativa and Senna alata seed extracts were prepared using ethanol, methanol and aqueous solvents in order to obtain bioactive compounds. Both ethanol and methanol yielded big amounts and high percentage of extracts, making them the most efficient solvents. However, the present study revealed that ethanol extraction in general afforded the highest yields of of bioactive compounds in both plant species. According to previous studies [17,21], the choice of solvent

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definitely influenced yield and composition of the extracts. Chemical constituents examination determined various phytonutrients present in the two plants such as alkaloids, flavonoids, saponins, tannins, phenolics, and terpenes that make plants to possess therapeutic values. For instance, Nigella sativa possess Thymoquinone while Senna possess Aloe-emodin- a compound which exhibits anti bacterial, anti-inflammatory, and anti-oxidant properties as pointed out in literature [22]. The extracts antibacterial activity was determined using both the Agardiffusion and the Bouillon dilution methods. Nigella sativa exhibited significant antibacterial against Staphylococcus aureus and considerable antifungal against Candida albicans, on the other hand Senna alata yielded considerable antifungal against Candida albicans and moderate antifungal against Aspergillus niger. While both extracts displayed moderate anti-inflammatory and antioxidant properties, the ethanolic extracts beneficial effects were found to be the most prominent in this present research as supported by similar past studies of [23].

4. Conclusion:

This study will differentiate and exhaustively analyze the therapeutic value of NS and SA through Phytochemical tests as well as the assessment of antimicrobial activity, anti-inflammatory features and antioxidant property. Comparing the different solvents with their respective extracts it was found that ethanol and methanol were the most effective solvents and ethanol gave the highest yields. Chromatographic studies Änthymoquinone from *Nigella sativa and Senna alata* were the phytochemicals Responsible for the medicinal value that has been found in plants. Varying inhibitory concentrations were observed for Antibacterial activity tests, were carried out and it was evident that the extract of Nigella sativa possessed a strong antibacterial activity against both *Staphylococcus aureus and Candida albicans and Senna alata* was found to have effective antibacterial activity against *Candida albicans and Aspergillus niger*. Inflammation and antioxidant activities were found to be positive in both the plants including the ethanolic extracts which marked the highest results. These findings endorse the ethnopharmacological application of NS and SW and it could be inferred that both are conducive as leads for the synthesis of novel drugs. More studies have to be conducted in order to identify particular phytochemicals, learn about their effects and maybe use them in today's pharmaceuticals. This present work is a very rich blend of indigenous practices together with contemporary research ideals and findings and stands as a foundation for subsequent analyses on the therapeutic values of these plants.

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Appendix :

Appendix 1 : Results of antimicrobial activity of *Nigella sativa and Senna alata* slant extracts against different microorganisms at different concentrations and extraction methods.

Extract	Concentration (mg/ml)	*Staphylococcus aureus (mm)*	*Bacillus subtilis (mm)*	*Escherichia coli (mm)*	*Pseudomonas aeruginosa (mm)*	*Candida albicans (mm)*	*Aspergillus niger (mm)*
N.sativa Ethanolic	50	14	13	12	10	16	14
N.sativa Methanolic	50	13	12	11	9	15	13
N.sativa Aqueous	50	11	10	9	8	13	11
Senna alata Ethanolic	50	12	11	10	9	14	12
Sennaalata Methanolic	50	11	10	9	8	13	11
Sennaalata Aqueous	50	10	9	8	7	12	10

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Appendix 2: The minimum inhibitory concentration values indicate the lowest concentrations (in mg/ml) of the extracts at which microbial growth was inhibited for each microorganism tested.

Extract	*Staphylococcus aureus (mg/ml)*	*Bacillus subtilis (mg/ml)*	*Escherichia coli (mg/ml)*	*Pseudomonas aeruginosa (mg/ml)*	*Candida albicans (mg/ml)*	*Aspergillus niger (mg/ml)*
N.sativa Ethanolic	6.25	6.25	12.5	25	3.125	6.25
N. sativa Methanoli	6.25	6.25	12.5	25	3.125	6.25
N.sativa Aqueous	12.5	12.5	25	50	6.25	12.5
Senmaalata Ethanolic	12.5	12.5	25	50	6.25	12.5
Senna alata Methanolic	12.5	12.5	25	50	6.25	12.5
Senna alata Aqueous	25	25	50	100	12.5	25

Appendix 3: Anti-inflammatory and antioxidant activity of *Nigella sativa and Senna alata* extracts for each tested microorganism.

Extract	Conc. (µg/ml)	Protein Denat. Inhib. (%)	Membrane Stabilization (%)	DPPH Rad. Scav. (%)	ABTS Rad. Scav. (%)
NS (Eth)	50	58.6 ± 2.1	62.3 ± 1.9	63.2 ± 2.3	60.5 ± 2.1
NS Meth	50	56.4 ± 2.0	60.2 ± 1.8	61.5 ± 2.2	59.1 ± 2.0
	100	66.7 ± 2.3	68.9 ± 2.2	69.8 ± 2.6	66.7 ± 2.4
	200	73.8 ± 2.7	76.5 ± 2.6	78.9 ± 3.0	75.1 ± 2.8
NS (Aq)	50	52.3 ± 1.9	57.1 ± 1.7	57.1 ± 2.0	55.3 ± 1.8
	100	62.8 ± 2.2	65.4 ± 2.1	65.3 ± 2.3	63.4 ± 2.2
	200	70.1 ± 2.5	72.6 ± 2.4	74.8 ± 2.7	71.9 ± 2.6
SA Eth)	50	54.2 ± 1.8	59.3 ± 1.7	59.4 ± 2.1	56.8 ± 1.9
	100	64.3 ± 2.1	67.5 ± 2.0	67.5 ± 2.4	64.9 ± 2.3
	200	71.6 ± 2.4	74.8 ± 2.3	76.2 ± 2.8	73.7 ± 2.7
SAMeth	50	52.8 ± 1.7	57.8 ± 1.6	57.9 ± 2.0	55.3 ± 1.8
	100	62.5 ± 2.0	65.9 ± 1.9	65.8 ± 2.3	63.4 ± 2.2
	200	70.2 ± 2.3	72.1 ± 2.2	74.3 ± 2.6	72.1 ± 2.5
SA Aq	50	50.1 ± 1.6	55.4 ± 1.5	54.6 ± 1.9	52.4 ± 1.7
	100	60.2 ± 1.9	63.7 ± 1.8	63.1 ± 2.2	60.9 ± 2.0
	200	68.4 ± 2.2	70.5 ± 2.1	71.5 ± 2.5	69.8 ± 2.0