

ANALYTICAL STUDY ON PERFORMANCE AND CHARACTERIZATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT: This examination was completed to distinguish the phytochemicals and in vitro antioxidant activity of methanol leaf extract of *Synsepalum dulcificum* (MSD). Standard conventions were utilized to assess the absolute phenols, all out flavonoids and all out antioxidants substance of the extract. Nitric oxide (NO), hydroxyl radical (OH), ABTS•+ and 2,2-diphenyl - 1-picrylhydrazyl (DPPH) radicals rummaging activity, inhibition of lipid peroxidation and the capacity of MSD to chelate ferrous particle too its reductive potential were additionally assessed. Elite Liquid Chromatography (HPLC) was utilized to affirm the nearness of polyphenols and carotenoids. Results indicated the nearness of flavonoids, saponins, terpenoids and cardiovascular glycosides in the extract.

KEYWORDS: Chromatographic, Linearity, Accuracy, Thermal.

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I. INTRODUCTION

As of late medicinal plants have involved a one of a kind situation among the treatment modalities of a wide assortment of infections. This probably won't be detached with the nearness of organically dynamic particles in such plants particularly atoms that have been demonstrated to have remedial possibilities in a few trial illness models. Such atoms incorporate flavonoids, tannins, steroids, terpenoids, saponins and so on. These atoms have been demonstrated to be answerable for the medicinal estimation of the plants in which they are found, including their antioxidant activity.

The antioxidant activity of a compound has been credited to different instruments viz: avoidance of chain inception, authoritative of change metal particle impetuses, deterioration of peroxides, counteractive action of proceeded with hydrogen reflection, reductive limit and radical searching capacity. Besides, The World Health Organization (WHO) expressed that over 80% of the populace in creating nations utilizes natural and other customary meds to treat their regular infirmities (WHO, 1998). Free radicals are exceptionally responsive substance species routinely created in the human framework by typical natural responses and furthermore by different exogenous elements. A portion of the radicals incorporate superoxide O₂⁻, nitric oxide (NO), peroxy (ROO), and hydroxyl (OH⁻) (Maritim et al., 2003). These ROS assume a significant job in degenerative or obsessive procedures, for example, maturing, malignancies, coronary heart illnesses, Alzheimer's ailment, neurodegenerative issue, atherosclerosis, waterfalls and aggravations.

II. LITERATURE REVIEW

Tim Tome (2019) This survey shows the Analytical Quality by Design (AQbD) idea, an augmentation of Quality by Design (QbD), which was presented in 2004 by the U.S. Nourishment and Drug Administration (FDA) and endorsed in 2005 by the International Conference on Harmonization (ICH). AQbD is a deliberate way to deal with technique advancement, controlling all phases of the scientific method life cycle. It incorporates a meaning of the expository objective profile (ATP), identification of basic strategy parameters or variables, and determination of basic technique traits (CMAs) or reactions. Screening and reaction surface experimental plans permit the acknowledgment of noteworthy variables and their improvement by measurable examination. The factor– reaction

relationship is portrayed by a scientific model, which can foresee the ideal reaction. Multivariate combinations of elements fulfilling the CMA necessities are introduced in the plan space or technique operable structure area (MODR), where vigorous strategy performance is guaranteed. This audit exhibits the hypothetical foundation of AQbD for strategy improvement and its work process through as of late distributed cases in which the AQbD idea demonstrated to be a solid and viable methodology. Diagnostic strategies created by utilizing the AQbD approach are profoundly vigorous, are effectively validated, have shorter run times, and are equipped for deciding a higher number of analytes in one run contrasted and the techniques created by the each factor in turn (OFAT) approach.

Ana Cristina de Mattos (2013) The goal of this work was to create and approve a quick superior fluid chromatography (HPLC) technique for the quantitative examination of fluorouracil (5-FU) in polymeric nanoparticles. Chromatographic examinations were performed on a RP C18 section with a versatile stage comprising of acetonitrile and water (10:90, v/v) at a stream pace of 1 mL/min. The 5-FU was distinguished and quantitated utilizing a photodiode cluster indicator at a wavelength of 265 nm. The technique was demonstrated to be explicit and straight in the scope of 0.1-10 µg/mL ($r = 0.9997$). The exactness (intra-and between day) was exhibited in light of the fact that the most extreme relative standard deviation was 3.51%. The strategy is hearty comparative with changes in stream rate, segment and temperature. The breaking points of detection and quantitation were 10.86 and 32.78 ng/mL, individually. The technique satisfied the necessities for dependability and plausibility for application to the quantitative investigation of 5-FU in polymeric nanoparticles.

Shengsheng Zhu (2013) An isocratic, delicate and stability-showing superior fluid chromatographic (HPLC) strategy for partition and assurance of the related substances of micafungin sodium was created. The chromatographic partition was accomplished on Agilent Zorbax SB-C18 section (250 × 4.6 mm, 5 µm). Forced degradation study affirmed that the recently created technique was explicit and specific to the degradation items. The performance of the strategy was validated by the present ICH rules for specificity, linearity, exactness, accuracy and robustness. Relapse examination demonstrated relationship coefficient esteem more prominent than 0.999 for micafungin sodium and its six impurities. Utmost of detection of impurities was in the scope of 0.006%–0.013% demonstrating the high sensitivity of the recently created strategy. Exactness of the technique was built up dependent on the recuperation got somewhere in the range of 98.2% and 102.0% for all impurities. RSD got for the repeatability and middle exactness tests, was under 1.0%. The strategy was effectively applied to measure related substances of micafungin sodium in mass drugs.

PowarTrupti Ashok (2019) The target of this work was to create and approve an elite fluid chromatography (HPLC) technique for the quantitative examination of melphalan, an anticancer drug from lyophilized nano-suspension. Material and Method: Chromatographic division was accomplished by utilizing a turnaround stage C18 segment (150 mm × 4.6 mm, pore size 5 µm, Phenomenex). The portable stage was streamlined as acidic corrosive, water and methanol (1: 49.5: 49.5) with pH 4 at a stream pace of 1 mL/min. The melphalan was recognized and quantitated utilizing an UV finder at a wavelength of 254 nm. Result: The technique was demonstrated to be explicit and direct in the scope of 10-50 µg.mL⁻¹ with relationship coefficient of 0.9979 and was exact at the intra-day level as reflected by relative standard deviation, precise at recuperation rate 99.75±0.08 and strong to change portable stage and segment brand. The detection and quantization limits were 0.2956 µg.mL⁻¹ and 0.5874 µg.mL⁻¹, individually. The proposed technique could be beneficial in estimation of melphalan quantization in lyophilized nano-suspension structure within the sight of excipients. End: The technique was seen as straightforward, explicit, quick, exact, precise and reproducible. The strategy was effectively applied for assurance of the entanglement proficiency of melphalan from lyophilized nano-suspension and was found to 93.56 ± 4.32%.

Sudha T (2012) Analytical technique advancement pursued by strategy approval is a significant process in the drug disclosure. In spite of the fact that the drug shows great strength, absence of validated diagnostic technique won't enable the drug to go into the market. This is to guarantee the quality and security of the drug. The primary target of this audit is to give a thought regarding the old and novel systems accessible for the examination of drugs in their crude material and detailed structures, check the stability of the drugs within the sight of the excipients and different pressure conditions experienced during their time span of usability period. The audit work puts a light on the hyphenated strategies for the examination and polluting influence profiling of drugs like LC-MS-MS, LCNMR-MS, GC-MS and LC – MS. This survey additionally manages the bioanalytical strategy advancement for the quantitative assurance of the drugs in the different organic grids. It additionally gives a way to decide the organic security of the drugs by managing the SIAMs (stability demonstrating measure techniques).

III.MATERIAL AND METHODS

Chemicals

Acetonitrile, formic acid, gallic acid, chlorogenic acid, ellagic acid and caffeic acid were purchased from Merck (Darmstadt, Germany). Quercetin, quercitrin, isoquercitrin, rutin, catechin, epicatechin and kaempferol, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, gallic acid, ascorbic acid, quercetin and Folin-Ciocalteu reagent were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Plant material and extraction

S. dulcificum leaves were collected from a farmland in Iseyin, Oyo-state in South-Western Nigeria. The leaves were identified and a voucher number- UIH-22457 was obtained for the leaf at Botany Department University of Ibadan, Nigeria. The leaves were air-dried for three weeks and pulverized. 700g of the pulverized sample was extracted in 80% methanol by maceration for 72 hours. The methanol extract was concentrated in a rotary evaporator and lyophilized. A total yield of the extract (98.7g, 14.1%) was obtained and preserved for further use.

Statistical analysis

Every statistical analysis were performed utilizing Microsoft exceed expectations bundle 2013 form. Results are communicated as mean± standard deviation. Independent sample t-test was utilized for data analysis. Linear regression analysis was utilized to figure the IC50 esteems.

IV. RESULTS AND DISCUSSION

Prior to the advent of modern drug herbs and natural items was the mainstay of medications around the globe. The phytochemicals present in regularly devoured plant nourishments are ordinarily non-poisonous and have the potential for averting chronic maladies. The plant extracts incorporate high centralization of flavonoids and phenolic mixes. As powerful cancer prevention agents, flavonoids are particularly significant for insurance against human infections. The numerous properties of these phytochemicals have made them progressively alluring, as they can modulate different parts of maladies like lipid peroxidation associated with atherogenesis, thrombosis, carcinogenesis, hepatotoxicity and an assortment of sickness conditions (Tiwari, 2001). Table 1 shows the phytochemical structure of the methanol concentrate of *S. dulcificum* leaves. Flavonoids, saponins, terpenoids and cardiovascular glycosides were available in the concentrate. Our outcome as introduced in table 2 likewise indicated that the all out phenols and all out flavonoids were evaluated to be 58.67 mg/g TAE and 13.01 mg/g QE separately.

Table 1: Phytochemical Screening of Methanol Leaf Extract of *Synsepalum Dulcificum*

Phytochemical	Result
Saponins	+ve
Tannins	-ve
Flavonoids	+ve
Alkaloids	-ve
Terpenoids	+ve
Anthraquinones	-ve
Cardiac glycosides	+ve
Steroids	-ve

The assessment of the antioxidant activity of medicinal plants has become a key component of the procedures utilized in disentangling the remedial possibilities of such plants.

Scientists opined that no single test precisely mirrors the component of activity of every single radical source or all antioxidants in an intricate framework (Prior et al., 2005) and that at least two strategies ought to be utilized so as to assess the complete antioxidant activity (Ilhami et al., 2005) because of different oxidative procedures.

DPPH radical rummaging activity is normally used to gauge the in-vitro antioxidant activity of characteristic mixes or plant extracts (Philips et al., 2010).

As appeared in figure 1 The DPPH rummaging activity of methanolic concentrate of *Synsepalum dulcificum* leaves contrasted well and tannic corrosive at all the fixations tried. At the most noteworthy fixation 400µg/ml the concentrate demonstrated 91.84% inhibition while the standard antioxidant indicated 93.53% inhibition of DPPH radical. The outcome likewise indicated that the concentrate showed a dose-dependent inhibition of DPPH radical.

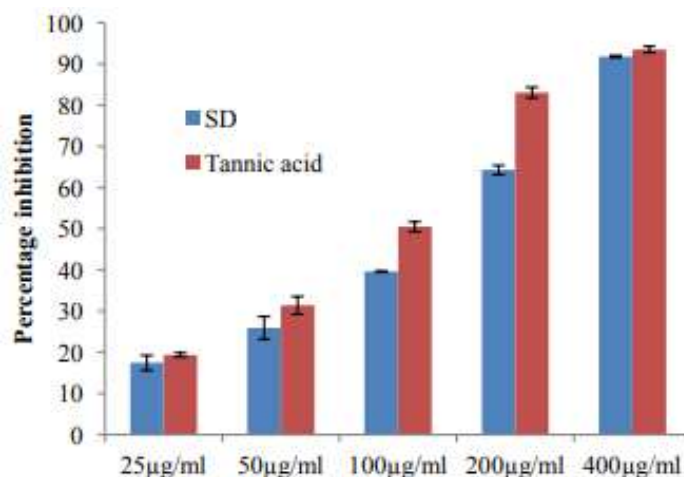


Fig. 1: Percentage DPPH Radical Inhibition of Methanol Leaf Extract of SD

Nitric oxide is a free radical delivered in mammalian cells, engaged with the guideline of different physiological procedure including neurotransmission, vascular homeostasis, antimicrobial and antitumor exercises. Be that as it may, overabundance creation of NO is related with a few sicknesses. It is fascinating to create intense and particular inhibitors of NO for potential restorative use have solid reducing power (Amin and Razieh, 2007). The outcome in figure 2 shows that the methanol extract of *Synsepalum dulcificum* leaves demonstrated a calculable reducing power when contrasted with quercetin which was utilized as the standard antioxidant. At 400µg/ml the extract and standard indicated absorbance estimations of 1.21 and 1.80 individually.

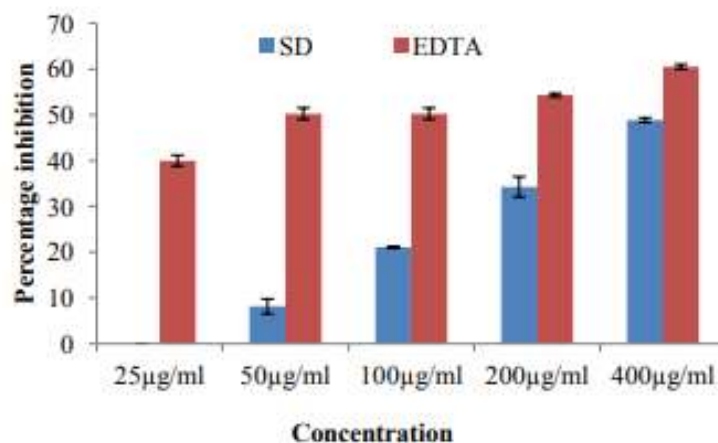


Fig. 2: Percentage Iron Chelating Activity of Methanol Leaf Extract of SD

HPLC/DAD unique mark ought to be considered to assess the nature of home grown medications everywhere throughout the world considering the way that numerous constituents are available in home grown prescriptions and its item (Coppen et al., 1983). Our outcomes as appeared in tables 2 show that the extract has a significant level of polyphenols in the request flavonoids (85.96 mg/g/8.58%) > phenolic acids (63.5 mg/g/6.33%) > tannins (23.87 mg/g/2.38%).

Table 2: Carotenoids Compositions of Methanol Leaf Extracts *Synsepalum Dulcificum*

Carotenoids	<i>S. dulcificum</i> mg/g
Tocopherol	2.06 ± 0.01 ^a
β-Carotene	2.93 ± 0.04 ^a
Lycopene	0.57 ± 0.01 ^a

Among the flavonoids rutin was the most rich pursued by quercetin, isoquercitrin, quercitrin and kaempferol in a specific order. Ellagic corrosive is the most plentiful among the phenolic acids present in the extract pursued by chlorogenic corrosive, caffeic corrosive, and gallic corrosive in a specific order. For tannins our outcome likewise shows that the extract contains a more elevated level of epicatechin than catechin. The nearness of carotenoids was additionally settled in the extract. Figure 3 shows the chromatogram demonstrating the pinnacles of the polyphenols that were identified in the extract. HPLC is a delicate, exact and explicit strategy for the division and recognizable proof of phenolic acids, flavonoid glycosides and aglycones in plants.

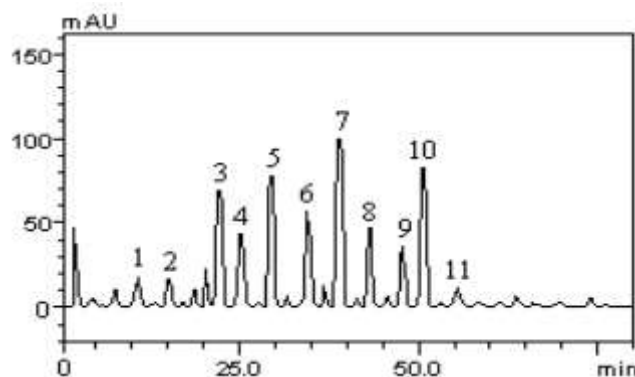


Fig. 3: HPLC Chromatogram of Methanol Leaf Extract of Synsepalum Dulcificum

V. CONCLUSION

From our examination it could be securely inferred that the methanolic extract of *Synsepalum dulcificum* leaves has considerable in vitro antioxidant activity and mineral substance. This is a reasonable pointer to the probability of the plant having potential medical advantages particularly in infections in which oxidants have been built up as the guilty party.

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